AN ANATOMICAL STUDY OF THE GASSERIAN GANGLION, WITH PARTICULAR REFERENCE TO THE NATURE AND EXTENT OF MECKEL'S CAVE

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EIGHT FIGURES

Late in the fall of 1920, when Dr. C. M. Van Allen was working on his new method of injecting the Gasserian ganglion by way of the orbit, he was confronted with the problem of determining the exact distribution which could be expected with material injected into the spaces about the ganglion. As this would be determined largely by the extent and nature of Meckel's cave and the subarachnoid space, he sought in the literature for information relative to the more exact anatomy of the ganglion, and found that, with the exception of the rather cursory discussion of the subject that was found in any of the standard text-books on anatomy, there had been little or no work published on the subject.

Because of this lack of literature, a study was undertaken originally, with the idea of publishing it in conjunction with his paper on orbital puncture. It was soon seen, however, that the problem was rather more difficult than originally anticipated and that it would be impossible to complete it satisfactorily in time.

The usually accepted definition of the term Meckel's cave is very brief. It is usually considered to be a space between the two layers of the dura mater at the tip of the petrous portion of the temporal bone. In this interval lies the Gasserian or semilunar ganglion. None of the descriptions indicate whether the space is an actual one filled with fluid or

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merely a potential space completely occupied by the ganglion, nor is any mention made of the inclusion in the cavity of any of the other meninges. Our object, then, has been to clarify the finer details of the anatomy of the region with special reference to any possible connections which might exist between the cavity itself, if any such existed, and the other meningeal spaces of the brain.

MATERIAL AND METHODS

After a survey of the available material and the information desired, it was decided to use two methods of approach.

The first method consisted in the injection of India ink into the subarachnoid space and, after proper fixation, in carefully dissecting the ganglion area to expose the extent of the subarachnoid relations. For this purpose we used foetal heads from stillbirths. These were obtained as soon as possible after delivery and injected at once. The injections were made with an ordinary Luer syringe and the subarachnoid space was reached through the anterior fontanelle. Care was taken to use little or no pressure, in order that there would be as little disturbance of the normal relations as possible. Following injection the heads were placed in a 10 per cent formalin solution and allowed to remain until thoroughly fixed. The calvarium was then removed, and before any dissection of the brain tissue was attempted the heads were again placed in the formalin solution for several days to be sure that complete fixation had been obtained. A careful dissection of the area involved was then done in the usual way.

The second method involved a microscopic study of a series of sections through the trigeminal region. We encountered several difficulties in getting our sections, chief of which was that of decalcification.

The most successful series was obtained in the following manner. A foetus which was born at seven months and lived but a few hours was obtained within three or four hours after death. The foetus was decapitated and the head placed at once in a freezing mixture until frozen solid. This was done

so that there would be no distortion produced in getting out the blocks. The desired blocks were then sawed out, trimmed, and put in Zenker's solution for fixation. Following fixation they were placed in a standard phloroglucin decalcifying solution, where they were allowed to remain two weeks. We had found with our earlier work that the bone of the petrous portion of the temporal was very dense and that the decalcifying solution worked quite slowly. The blocks were then dehydrated in the usual way, embedded in paraffin, trimmed up, and serial sections made. One block was cut in a frontal plane beginning from the upper or cephalic surface of the block; the other was cut in a transverse plane beginning anteriorly. (The planes of section are related to the body.) All sections were cut 20 μ in thickness. They were then mounted and stained with haematoxylin and erythrosin. The series, while too thick for detailed analysis of cell structure, served excellently to show tissue relations and the cytological definition was very satisfactory.

In order to get a better appreciation of relations as shown by our sections, we made a wax reconstruction, using plates 2 mm. in thickness. Drawings were made with the aid of an Edinger projection apparatus at a magnification of 50 diameters. In piling the plates the medial posterior corner of the block was used as a landmark. We had, previous to sectioning, squared this as carefully as possible, and it served our purpose very satisfactorily. After piling, the plates were smoothed and modeled in the usual way and the model painted.

MICROSCOPIC ANATOMY

A study of the microscopic sections prepared in the above manner makes possible a fairly detailed account of the meningeal relations of the Gasserian ganglion together with the nerve roots of the ganglion. At the point where the root of the fifth nerve crosses the cephalic crest of the petrous portion of the temporal bone it is found to consist of many small bundles of nerve fibers, each with a pial investment.

The nerve bundles lie rather well separated from each other with a considerable interval between the fascicles. These intervals are definitely continuous with the basal cisterns which lie in the posterior fossa of the skull. The margin of the aperture by which the fifth nerve root pierces the dura

ABBREVIATIONS

ab., abducens n.
cp., carotid plexus
cs., cavernous sinus
d., dura
Gg., Gasserian ganglion
ica., internal carotid artery
mc., Meckel's cave
mn., mandibular n.
mr., motor root
mx., maxillary n.
oc., oculomotor n.
oph., ophthalmic n.
pa., pia-arachnoid
sbs., subarachnoid space
sr., sensory root
tr., trochlear n.

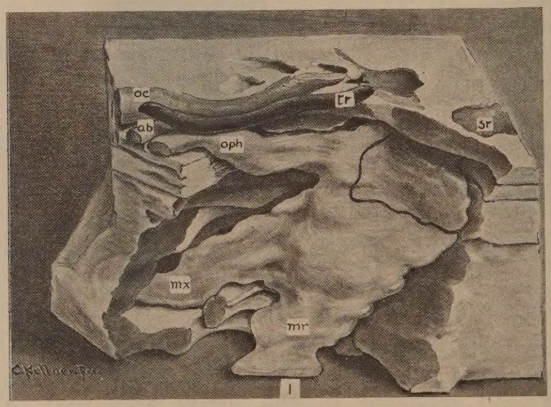


Fig. 1 An anterolateral view of a model of the left Gasserian ganglion in situ in a seven months' foetus. × 12. The black line shows the lower limit of Meckel's cave.

is marked by a clearly defined dural sheath. While the sections in this region do not clearly show an arachnoid, owing probably to a destruction of the delicate membrane in sectioning, its presence farther along the root indicates clearly that it is existent about the root bundles themselves (fig. 3). It may be said, then, that the root of the fifth ganglion crosses the crest of the petrous portion of the

temporal bone surrounded by a definite pia-arachnoid and entering a definite interval between the inner and outer layers of the dura. This dural space is usually given the name of Meckel's cave or space. As the nerve root turns caudally over the anterior face of the bone the fascicles become more compact with the interfascicular spaces considerably reduced. These spaces are definitely continuous with the intervals about the root as it crosses the crest of the temporal bone.

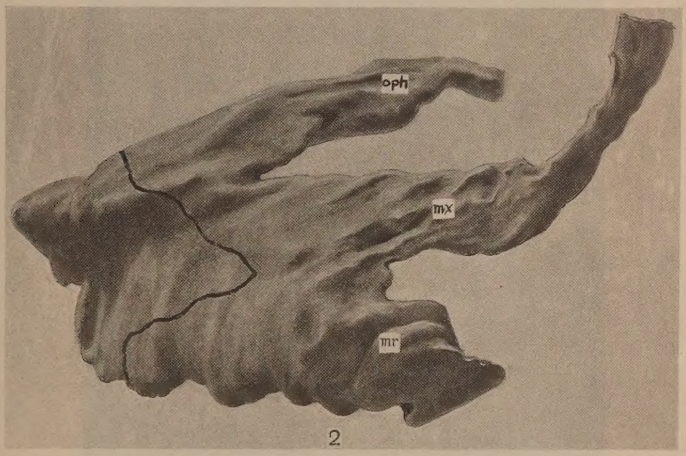
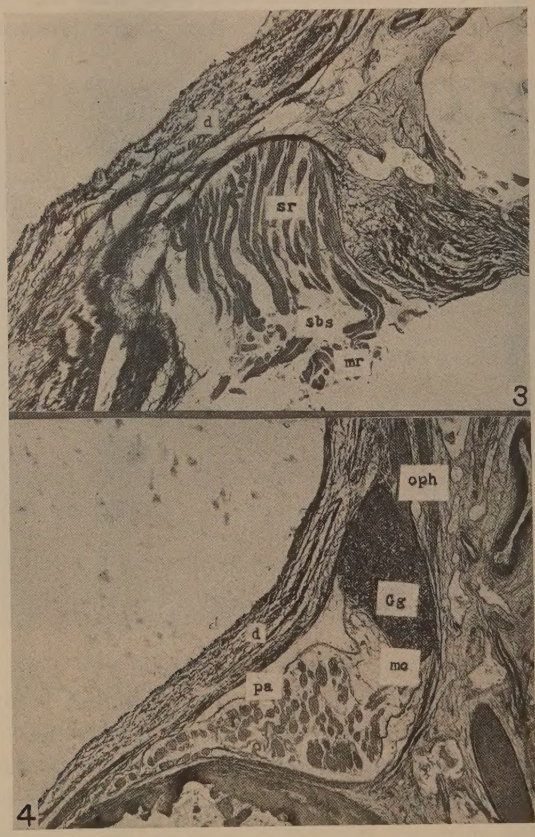


Fig. 2 A posterior view of the ganglion and its main branches after removal from the model shown in figure 1. × 14. The black line shows the lower limits of Meckel's cave behind the ganglion.

Owing to the greater fixation of the nerve root, as one progresses deeper into the cave the arachnoid is preserved intact and can be seen as a well-defined membrane surrounding the nerve root and attached distally to the proximal face of the Gasserian ganglion on both its superficial and deep aspects (fig. 4). A considerable interval was discovered between the pia-arachnoid and the dura on the anterior face of the root as shown in figure 4. Furthermore, with the appearance of the ganglion the close connection established between the ganglion tissue itself and the dura was obvious.

The distal faces of the ganglion were completely invested by the dura which was so closely adherent to its periphery that the presence or absence of a pia-arachnoid investment was impossible to determine. The study of serial sections would seem to indicate that the majority of the ganglion itself was inclosed in a fused dura and pia-arachnoid. The anterior



Figs. 3 to 7 Photomicrographs of critical sections from the series upon which the model was reconstructed. × 24.

Fig. 3 The meningeal investments of the root of Gasserian ganglion.

Fig. 4 The upper third of the ganglion showing Meckel's cave and the subarachnoid space about the root.

face of the ganglion throughout its entire extent was closely covered without any space or apparent possibility of a potential space by the combined dura and pia-arachnoid.

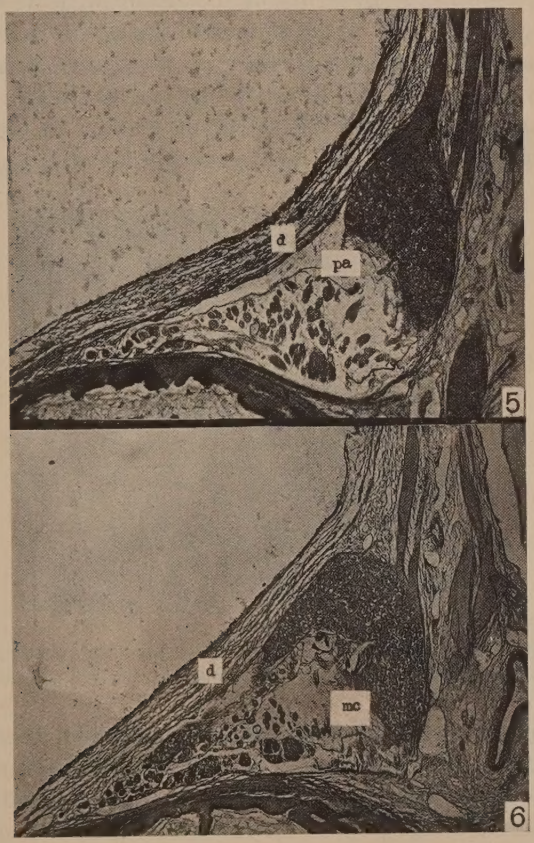


Fig. 5 A section showing the sympathetic connections of the ophthalmic.
Fig. 6 The middle third of the ganglion.

On the posterior surface of the ganglion the conditions were somewhat different. In the first place, it is on this aspect of the ganglion that the motor root of the fifth runs. As will be seen in figure 7 the motor root traverses a valley

on the deep surface of the ganglion. It is surrounded by pia with a definite interval between pia and arachnoid. This interval is obviously directly continuous with the spaces about the entire root. The presence of the valley with its contained motor root and a few of the longer fibers of the sensory root continues down the deep surface of the ganglion as far as the junction of the middle with the distal third. At this point as on the anterior face, the meningeal investment of the ganglion is densely related to the ganglion surface. The line

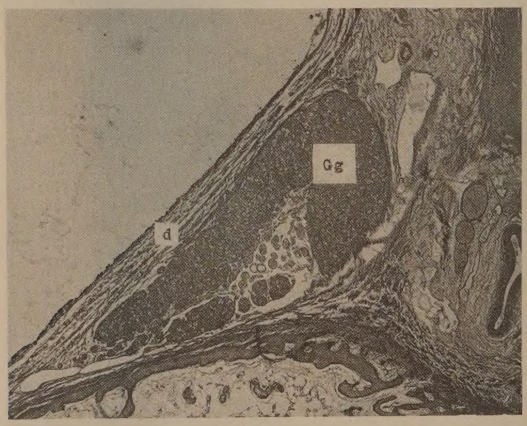


Fig. 7 The junction of the middle with the lower third, showing the lower limit of the subarachnoid space behind the ganglion.

of this firm attachment is shown in figure 1 on the anterior surface of the ganglion and in figure 2 on the posterior face. The area proximal to the heavy black line constitutes that portion of the ganglion and its roots to which the meningeal coverings are not densely adherent. It, therefore, is the only portion of the ganglion and root about which there can be definitely shown to be any well-defined space. This space is a definite subarachnoid space continuous with the basal cisterns of the posterior fossa of the skull. From the above brief description it will be noted that this subarachnoid space does not extend distally over the anterior face of the ganglion

beyond the point of entrance of the sensory root. On the posterior surface the same statement is true except that the sensory roots here progress further distally before they enter the ganglion tissue. Hence we find that the proximal two-thirds of the posterior surface of the ganglion is separated from the dura by a definite subarachnoid space.

GROSS ANATOMY

The findings resulting from the injection of India ink into the subarachnoid spaces quite parallel the microscopic find-

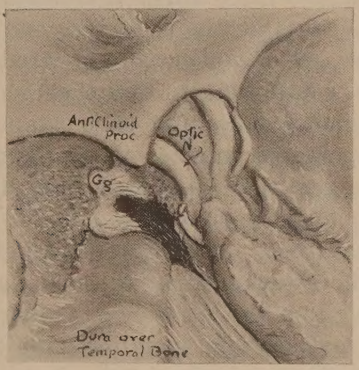


Fig. 8 A dissection of the left Gasserian ganglion in a seven months' foetus in which the subarachnoid spaces had been filled with India ink. The deposit of ink on the anterior surface of the root of the ganglion is clearly shown. × 2.

ings. Gross dissection of the infant heads so prepared showed a black precipitate of India ink in the subarachnoid space of the cisterna pontis. This black precipitate could be traced anteriorly about the sensory root of the fifth nerve as it crossed the crest of the petrous portion of the temporal bone. As the nerve root dipped downward to enter the ganglion the carbon particles completely surrounded it, stopping short as it reached the anterior surface of the ganglion, but pushing down behind the ganglion until it reached the distal third (fig. 8). Obviously the India ink in the cisterna pontis worked its way forward over the crest of the petrous portion

of the temporal bone to enter the subarachnoid space in Meckel's cave. The extent to which that space was occupied by carbon particles corresponded exactly with the extent of the space as determined by the microscopic study. Since considerable care was taken in the pressure exerted in the process of injection of India ink, there is little likelihood that any delicate curtain of pia-arachnoid across the entrance to Meckel's cave could have been ruptured. In any event, even though this had resulted, the extent of injection indicates a readily accessible pathway from this space about the ganglion into the cisterna pontis. It could also be shown with the dissecting needle that the root was composed of separate bundles of fibers, all of which were covered with the carbon particles. This would indicate that they occupied a common space. The manner in which the cave extended much farther distally on the posterior surface of the ganglion was readily shown when the ganglion was lifted from its bed. The root fibers and the coloring extended to a point quite low on the midline of this surface. In no case did the injected material reach beyond the attachment of the meningeal coverings to the ganglion on either the anterior or posterior surface. We find, then, that the Gasserian ganglion with its roots lies in an interval between the two layers of the dura, which covers the anterior face of the petrous portion of the temporal bone. On the anterior face of the ganglion the dura with a possible involvement of the pia-arachnoid is densely adherent for practically its entire extent. On the posterior surface it is adherent only on its distal third. From the line of fixation proximally the ganglion tissue and the nerve roots lie in a definite subarachnoid space directly continuous over the crest of the temporal bone with the cisterna pontis.

In the course of the study one or two other pertinent observations were made. In the first place, the ophthalmic and possibly the maxillary divisions of the fifth are connected by definite nerve-fiber bundles with the sympathetic plexuses of the internal carotid artery and cavernous sinus. While these connections occur outside of the ganglion, they are,

nevertheless, less than a millimeter from it. It would be difficult, therefore, to conceive of any radical procedure involving the ganglion which did not in a certain per cent of all cases traumatize the connection. It is suggested that this may be an explanation of the trophic disturbances which occasionally result in the eye from operations on the Gasserian ganglion. A certain color is given this statement by some findings in the surgical field in recent years. It has been found for example that the trophic disturbances following section of the median nerve may be very considerably improved by stripping the adventitia from the blood vessels supplying the region of the palm of the hand. Such a procedure would obviously destroy the vasomotor mechanism and possibly the afferent arc of a sympathetic reflex circuit.

Again it was found that the geniculate ganglion of the seventh nerve in the seven months' foetus lies about 5 mm. lateral to the ganglion on a level with the inferior portion. At this stage of development the ganglion is quite superficial, lying in a funnel-shaped depression on the anterior face of the petrous portion of the temporal bone. From this ganglion the great superficial petrosal nerve runs directly medially under the dura and behind the mandibular division of the trigeminal. Beyond this point it turns anteriorly and medially to join the deep petrosal and, as the pterygoid nerve, courses through the pterygoid canal to join the sphenopalatine ganglion. Here again it is obvious that radical procedures involving the Gasserian ganglion can hardly be carried out without injury to the greater superficial petrosal nerve. Through it injuries can unquestionably be communicated to the geniculate ganglion itself. It is not difficult to conceive of sufficient trauma thus being communicated to the ganglion to produce the facial palsies which follow a portion of all Gasserian ganglion operations.

CONCLUSIONS

In view of the extensive subarachnoid space surrounding the root of the fifth nerve and its direct connection with the cisterna pontis, it can be seen that any procedure involving the injection of alcohol or other toxic substances into the ganglion is necessarily fraught with considerable danger. This is particularly true because of the way that the subarachnoid space covers such a large portion of the posterior surface of the ganglion. The dense dural covering of the ganglion tissue also adds to the danger, for it makes certain that if the injecting needle does not reach the subarachnoid space directly, as is highly probable, as material is injected into the ganglion tissue, it will burst into the subarachnoid space, since this is the point of least resistance. Van Allen found this to be true in all cases where more than 0.5 cc. of material was injected into the ganglion. The danger of toxic substances is shown in the transient disturbance of the function of the third, fourth, sixth, and seventh nerves in case V of Van Allen's series.

This connection with the basal subarachnoid cisterns which makes the injection of alcohol dangerous suggests the possibility of using Van Allen's approach as a means of lavaging the basal cisterns as has been suggested by W. P. Eagleton in his article on suppurative meningitis.

The sympathetic connections of the ophthalmic division occur outside the ganglion, but so close to it that any procedure involving the ganglion is sure to produce trauma to them in a rather high percentage of cases, and this seems to us a possible explanation of the trophic disturbances that occur in the eyeball following a certain per cent of operations on the ganglion. It would seem from our studies that injections into the ganglion and puncture by Van Allen's route would be free from this danger because of the protection offered by the dense covering of the ganglion tissue in that area.

The facial palsies that occur following a portion of all ganglion operations would seem to have a somewhat similar

explanation. When one notes the intimate relation of the greatest superficial petrosal nerve to the dura and to the mandibular division of the ganglion, it will be appreciated that the stripping up of the dura from the surface of the petrous bone and the removal of the ganglion will in practically every case produce trauma to the greater superficial petrosal nerve and is certain in a definite per cent of those cases to injure the geniculate ganglion and the facial nerve.

SUMMARY

1. The meningeal covering of the ganglion is densely adherent on the anterior surface and on the distal third of the posterior surface.

2. On the proximal two-thirds of the posterior surface the ganglion is separated from the dura covering the petrous bone by an arachnoid membrane and a definite subarachnoid space that communicates freely with the cisterna pontis.

3. There are sympathetic connections with the ophthalmic divisions about 1 mm. anterior to the ganglion.

4. The greater superficial petrosal nerve runs medially directly under the dura on the petrous bone and then immediately behind the mandibular division of the fifth.

5. Injection of any toxic substance into the ganglion is fraught with considerable danger.

6. Transorbital puncture offers a means of draining or lavaging the basal subarachnoid cisterns.

7. Ulcers of the cornea following ganglion operation may be due to trauma to sympathetic connections of the ophthalmic division.

8. Facial palsies following ganglion operations may be due to injury to facial nerve indirectly by means of the greater superficial petrosal nerve and the geniculate ganglion.

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DESCRIPTION OF A SEX-INTERGRADE OPOSSUM, WITH AN ANALYSIS OF THE CONSTITUENTS OF ITS GONADS[1]

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ELEVEN FIGURES (TWO PLATES)

In the November, 1920, number of Science one of us gave a brief description of a sex-intergrade opossum and interpreted the specimen as a 'reciprocal free-martin,' that is, a zygotic male sterilized by female hormones. The gonads of this interesting individual have since been subjected to detailed study and analysis and have been compared with the adult sex glands, so that it is now possible to present a more complete account of the aberrant individual.

The animal was brought into the laboratory January 31, 1917.[2] It was a large animal, measuring 390 mm. in head-rump length and weighing 1750 grams. It was purchased as a male, for such it appeared to be, as judged from its general habitus.[3] On closer inspection is was found to have skin folds simulating a pouch, which, though small and misshapen (fig. 2), was yet better developed than in a normal adult male, in which a matting of hairs alone marks the pouch region. The retracted penis was of normal structure and dimensions. The scrotum was developed to normal proportions, but was empty.

[1] Contribution from the Department of Zoölogy, the University of Texas, no. 185.

[2] Part of a collection made by C. H. Heuser and Carl Hartman under the auspices of The Wistar Institute of Anatomy, Philadelphia, Dr. M. J. Greenman, director.

[3] In the previous communication (Hartman, '20) the head was erroneously described as favoring the female type. The head was fortunately preserved. It is of the male type.

On opening the abdomen a distinctly female genital tract, infantile in size, came to view (fig. 1). All of the parts of the Müllerian duct were represented: vagina, lateral vaginal canals (fig. 4), uteri, and Fallopian tubes (fig. 3). The infantile stage of the tract was reflected not only in its diminutive size, but also in the simple character of the mucosa, in the simple structure of the uterine glands (fig. 3), and particularly in the fact that the lateral vaginal canals were not yet patulous in their caudal portions (fig. 4) where they joined the median vaginal canal (cf. Hartman, '23).

It is thus apparent that the Müllerian ducts developed as in the female. But the influence of female hormones is likewise to be seen in the repression of the Wolffian ducts. The round ligaments of the uteri are normal in size (fig. 1) and therefore out of all proportion to the retarded genital tract. In the walls of the vagina on either side are seen tubes, somewhat coiled in a portion of their course. These tubes should, doubtless, be identified as Gärtner's ducts. There are, therefore, no vasa deferentia, only their homologues as they occur in the female.

Our specimen is thus seen to possess externally male sex characters (scrotum, penis, general habitus) and internally female sex characters, that is, a female genital tract. Thus far we find the exact opposite of the conditions that obtain in the free-martin of cattle (see table below).

The specimen likewise differs widely from the interesting type of mammalian sex intergrades described by Crew ('23 a). In the thirty-five pseudohermaphrodites studied by him, there is an intimate mixture of male and female accessory sexual apparatus, inasmuch as derivatives of both Müllerian and Wolffian ducts are found well developed in the same individual. The gonad is always distinctly a testis. External genitalia are imperfect, but more like those of the female than the male. There is never a scrotum.

Let us now examine the gonads of our hermaphroditic opossum.

THE GONADS

The gonads of the subject are, as far as we know, unique in the literature of sex intergrades in mammals.

From figure 1 it is seen that the gonads occupy the physiological position of ovaries and that their size is proportionate to the diminutive size of the genital tract.

From the middle of the organ to the hilus is a rete ovarii (figs. 7 and 10) quite like that which we find persisting throughout life in the opossum ovary. The most prominent structures of the gonads are, however, the rounded bodies (figs. 5, 7, and 10) that occupy the medulla of the organ about the rete. These bodies the senior writer (Hartman, '20) previously designated as testicular tubules of the cryptorchic type. We now know, however, that the bodies in question are not testicular tubules, but anovular Graafian follicles.[4] They are six times the diameter of testicular tubules and are spherical in shape or nearly so. Exactly comparable bodies, somewhat smaller in size (cf. fig. 10, magnified 50 diameters with fig. 11, $\times$ 100) occur in many normal adult ovaries where they often have a wide distribution. In one case many were seen collected at one point, as in figure 11. A comparison of figures 8 and 11 (from a normal ovary) with 7 and 10 (from the hermaphrodite) is sufficient to disclose the striking similarity.

These anovular follicles all consist of both granulosa and theca (figs. 7 and 8). The former gives the impression of being in a state of slow degeneration. More or less pycnotic nuclei are arranged peripherally and the cytoplasm is spongy and very coarsely reticular. Cell boundaries have for the most part disappeared. The granulosa is separated from the theca by a sharply defined basement membrane (figs. 7 and 8), consisting very definitely of connective-tissue fibers and nuclei. Outside these there is a definite theca interna of variable constitution and extent. In normal adult ovaries the theca of anovular follicles, as in figure 8, shows a tendency

[4] The results of our study of anovular follicles will be presented in an early number of this journal.

to form interstitial tissue.[5] In our hermaphrodite the theca contains typically large cells with rounded nuclei. Their cytoplasm, in contrast to that of the granulosa cells, stains well with orange G. The quantity of theca cells is subject to great variation in all of the anovular follicles.

Since we have determined that the large type of anovular follicles arises from the loss of the egg in the normal ovary, it is reasonable to suppose that in our sex intergrade they arose in the same way and that they are the modified remains of true Graafian follicles.

In speculating upon the source of anovular follicles of our specimen it is important to note that, according to Allen ('04) and others, Graafian follicles may arise as temporary structures from the medullary cords (sex cords of Allen) of the first proliferation from the germinal epithelium. Valentin-Pflüger egg tubes need not have arisen, therefore, to account for the follicles in question.

Other structures of the gonads are cell strands which we have called medullarly cords (figs. 5, 7, and 10) from their great similarity to medullary cords of embryos. They are branching structures, rather extensively distributed between the anovular follicles and the surface. They can, in places, be traced close to or even in contact with the superficial layer of cells, which are mostly squamous cells resembling peritoneum rather than germinal epithelium. Such cell cords are sometimes met with in adult ovaries,[6] as shown in figures 6 and 9. In the latter the cell cords are found in a process near the hilus of the ovary. Here the cords may be traced to the germinal epithelium, a point of interest and a subject of future inquiry in the light of Edgar Allen's ('24) work on postpubertal oogenesis in the mouse.

[5] The origin of interstitial tissue from the theca interna of the opossum ovary has been established by studies in this laboratory.

[6] Woerdeman ('20) recently described a human ovary which contained sex cords apparently similar to those here described, but many of them contained one or several ova. The author speaks of the cells composing the cords as 'bright,' meaning presumably that in contrast to the surrounding stroma they fail to take on a cytoplasmic stain. In this paper, also, the cords are like those here described by us.

It is true that in the intersexual opossum gonad there was no evidence of continued proliferation of sex cords, for no mitoses are to be seen in them. Yet they recall the sex cords described by Crew ('23 c), Fell ('23), and Gatenby and Brambell ('24) as proliferating from the peritoneal covering of diseased ovaries of hens in the process of transforming from female into male individuals.

The 'sex' of the gonad cannot from the foregoing description be determined with certainty. In either case, whether the gonad is a testis transformed into an ovary by female sex hormones, as would occur in a 'reciprocal free-martin,' or whether the organ is an ovary in which for some reason the second proliferation from the germinal epithelium was modified, Pflüger's egg tubes would be absent. The reader is referred to the instructive diagrams of Willier ('21, fig. 1, p. 67) in which the change of the free-martin gonad from the indifferent stage is clearly depicted. The gonad described by us is but a step in advance of his figure B, namely, in the differentiation of the Graafian follicles.

It should finally be pointed out that the two gonads were identical in contrast with avian hermaphrodites, in which there is always an ovary or ovotestis on the left side (Hartman and Hamilton, '22; Benoit, '23) whether the latent testis of the right side develops or not. Lateral true hermaphrodites have also been described in numbers. The ovary may be either on the right side (Corner, '21) or the left side (Kingsbury, '09).

DISCUSSION

The foregoing facts have been presented for their own sake and for their intrinsic interest. It might be profitable, however, briefly to speculate upon the causes that brought the sex intergrade into being.

As noted above, the animal was previously classified as a 'reciprocal free-martin' (Hartman, '20); that is, a zygotic male sterilized by a sister's sex hormones in utero—a contention which Lillie ('23) characterizes as based on the 'slenderest evidence.'

Space will not be consumed with a review of the facts presented by Lillie ('17, '23) and his students (Chapin, '17; Willier, '21; Bascom, '23, and Bissonnette, '24) in their analysis of the free-martin of cattle. The hormone theory of the free-martin may be considered established by the beautiful work just cited as well as by the observations of Keller and Tandler ('17).

It is not amiss, however, at this point to tabulate the chief characters of the free-martin of cattle with the corresponding characters of the opossum intersex studied by us:

Table of comparison between the free-martin of cattle and the sex-intergrade opossum ('reciprocal free-martin'?)

Free-martin	*Our Specimen*
1. Genetic female	1. Genetic (?)
2. Female habitus	2. Male habitus
3. Externally female	3. Externally male
4. Udder present	4. Defective pouch
5. Never a scrotum	5. Scrotum normal
6. Clitoris normal (female)	6. Penis normal (male)
7. Müllerian duct repressed	7. Müllerian duct developed in all its parts
8. Wolffian duct developed	8. Wolffian duct repressed
9. Vas deferens as in male	9. Gärtner's duct only
10. Inguinal ligaments	10. Round ligaments of uterus
11. Potential ovary becomes like testicle	11. Gonad like ovary
12. Pfluger's tubes absent through inhibition by hormones	12. Pfluger's tubes absent
13. Sex cells destroyed by male hormones	13. Sex cells absent
14. Sex cords become sterile tubules	14. Sex cords as in female
15. No Graafian follicles	15. Anovular Graafian follicles from sex cords
16. Rete as in testes	16. Rete as in ovary

The table is instructive. The antithesis between our animal and the free-martin of cattle is striking and would seem to justify the designation 'reciprocal free-martin' to the former.

Against the interpretation implied in the designation, however, we may urge certain conclusions reached by Lillie and his students.

Lillie ('23) contends first that the young female embryo of cattle does not produce sex hormones—only the male embryo does so. Presumably the female develops zygotically, undirected, without controlling sex hormones. In correlation with Lillie's theory, Bascom ('23) finds that only the male gonad produces interstitial cells in fetal life, the female gonad not until the Graafian follicles begin to undergo atresia.[7]

More conclusive evidence in the same direction is furnished by the condition of the opossum embryo at birth, that is, only about twelve days after fertilization. Dr. C. H. Heuser[8] has kindly examined for us the urogenital organs of three fetuses at the crucial stage: one embryo several hours before birth, and two pouch young, one and two days old, respectively. Doctor Heuser reports as follows:

"In the two older specimens (one- and two-day old pouch young) I find in the gonads an early differentiation of the medullary cords. Judging from the condition in pig, rat, etc., I believe that both specimens are females. The sex of the embryo near term cannot be determined and may be said to be in the indifferent stage. It would, therefore, seem that parturition marks the time of earliest sex differentiation in the gonads.

"The Wolffian body in the oldest specimen is still well maintained and must constitute the functional kidney at this time, since the metanephros is still rudimentary. The Müllerian ducts can be traced for some distance on the Wolffian body and they by no means approach each other. A considerable time must, therefore, elapse before fusion takes place."

[7] It seems gratuitous, however, to assume that because a certain condition prevails in cattle it also applies to other mammals. Allen, for example, found interstitial cells in the ovary of pig embryos at a much earlier stage than Bascom found them in cattle. It is conceivable that the condition may even be reversed in some forms. Nor is the embryonic history of interstitial tissue at all adequately known for any species; indeed, there is as yet no agreement among workers in this field for criteria by which to identify interstitial cells, to say nothing of their alleged incretory functions, as lately pointed out by Kingsbury ('24).

[8] Department of Embryology, Carnegie Institution of Washington, Baltimore.

From these observations the conclusion seems inevitable that our animal was not modified in its sexual development in utero, either through blood community with a sister embryo or through the hormones of the mother, unless one presuppose a long latent period or a storage of the sex hormones in question. The animal is, therefore, probably not a free-martin or its reciprocal.

The alternative is that the animal was born with an inherent defect. Two possibilities occur to us: the animal may have been a true hermaphrodite with two sets of gonads in which first the female, then the male gonads became prepotent. A survey of the literature on mammalian sex intergrades renders this unlikely. We prefer to picture the development of the specimen which we have described above as follows: The animal began as a female possessing XX chromosomes (Painter, '22). The gonads developed normally through the first proliferation of sex cords and elaborated sufficient female hormone to establish Graafian follicles and to direct the growth of the Müllerian ducts as well as the atrophy of the Wolffian ducts during the irritable stage of these organs. Something then happened in the animal which inhibited the proliferation of Pflüger's egg tubes and changed the second proliferation of the germinal epithelium to sex cords, as in the birds of Crew ('23 c), Fell ('23), and Gatenby and Brambell ('24). With Crew we may assume that after this change took place the external secondary sex characters, such as penis, scrotum, and skeleton, had entered the irritable stage and were directed by adequate male hormones from the sex cords in the male direction. It is not to be supposed, however, that more than a parallelism or certain analogy is intended between the cases of sex reversal in fowl and our present case, for the female of birds is heterozygous for sex, and hence sex abnormalities in birds and mammals would be conditioned by different mechanisms.

SUMMARY

1. A sex-intergrade opossum is described having the external appearance of a male, with normal penis and empty scrotum. Internally there is a distinctly female genital tract, with the Wolffian duct repressed as in the female.

2. The genital tract is infantile, although the animal is large and at least two years old.

3. In the gonads there is no trace of sex cells. The rete is of the female type. Large anovular Graafian follicles with thecae are grouped about the rete, and sex cords occupy the cortex. No characteristic interstitial cells can be found in the stroma. All of the structures found in these gonads may also be observed in normal adult ovaries of the opossum.

4. Inasmuch as only the first trace of sex differentiation can be detected in the opossum pouch young at birth, the animal in question can hardly be either a free-martin or a 'reciprocal free-martin,' but probably developed its hermaphroditic structure after birth.

5. An alternative interpretation considers the above-descibed animal a genetic female that underwent sex reversal somewhat like the fowls described by Crew and others. According to this view, the first proliferation of the germinal epithelium produced structures responsible for female hormones that controlled the development of the genital tract. The second proliferation later produced sex cords secreting male hormones which determined the male secondary sex characters.

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PLATES

PLATE 1

EXPLANATION OF FIGURES

1 The genital tract of the sex intergrade discussed in this paper (no. 366), dorsal view. The gonad (*g*), uterus (*ut*), and vagina are well shown. Threads, pulled sidewise, are tied to the ligaments of the bladder. The lateral vaginal canals cannot be seen. Natural size.

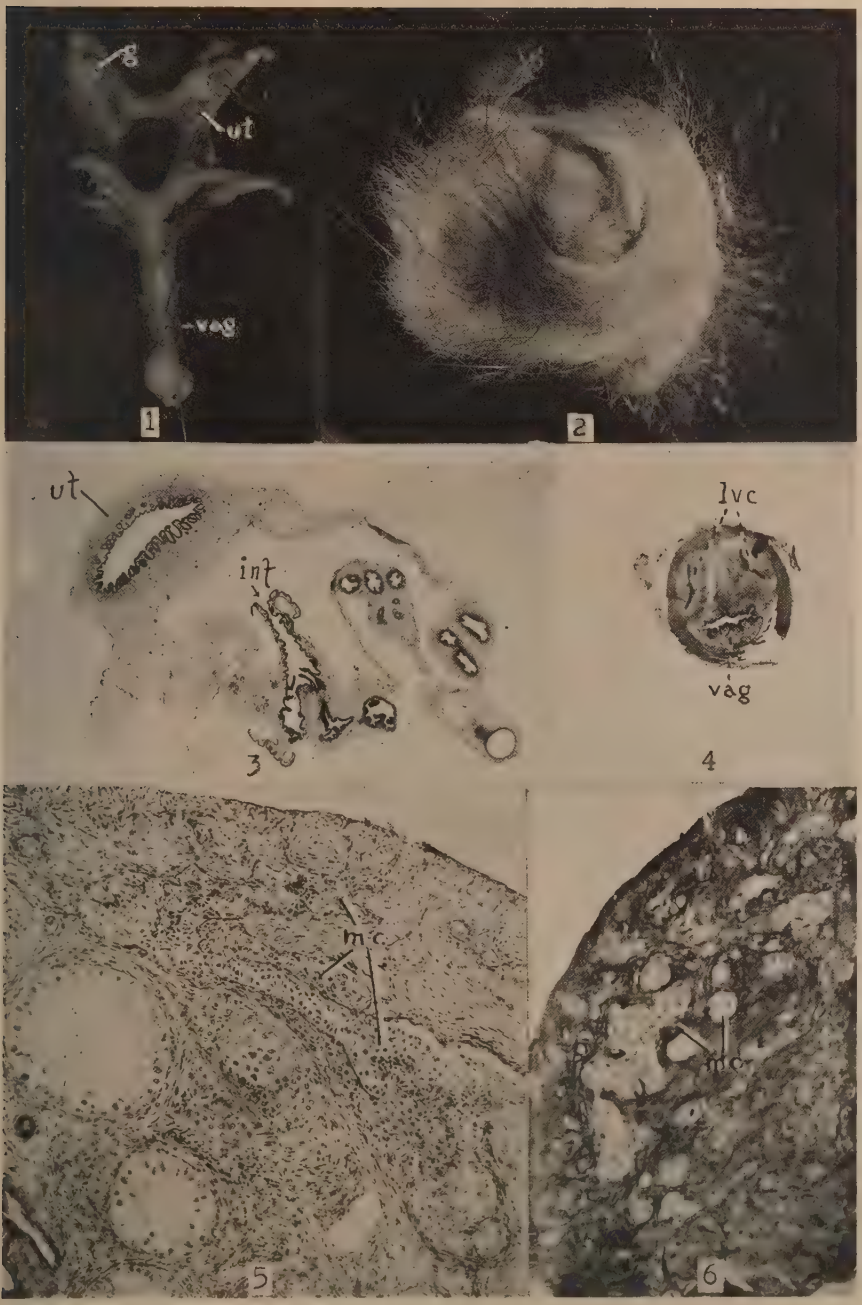
2 Rudimentary pouch of the animal. Natural size.

3 Section through uterus (*ut*), Fallopian tube, and infundibulum (*inf.*) × 8.

4 Section through median vaginal canal (*vag*) and caudal end of lateral vaginal canals (*lvc*). The latter disclose their infantile stage by virtue of their non-patulous condition. × 8.

5 Portion of gonad of sex intergrade, showing two anovular follicles and medullary cords (*mc*). × 100.

6 Portion of adult opossum ovary near hilus, showing medullary cords (*mc*). × 100.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vag
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PLATE 2

EXPLANATION OF FIGURES

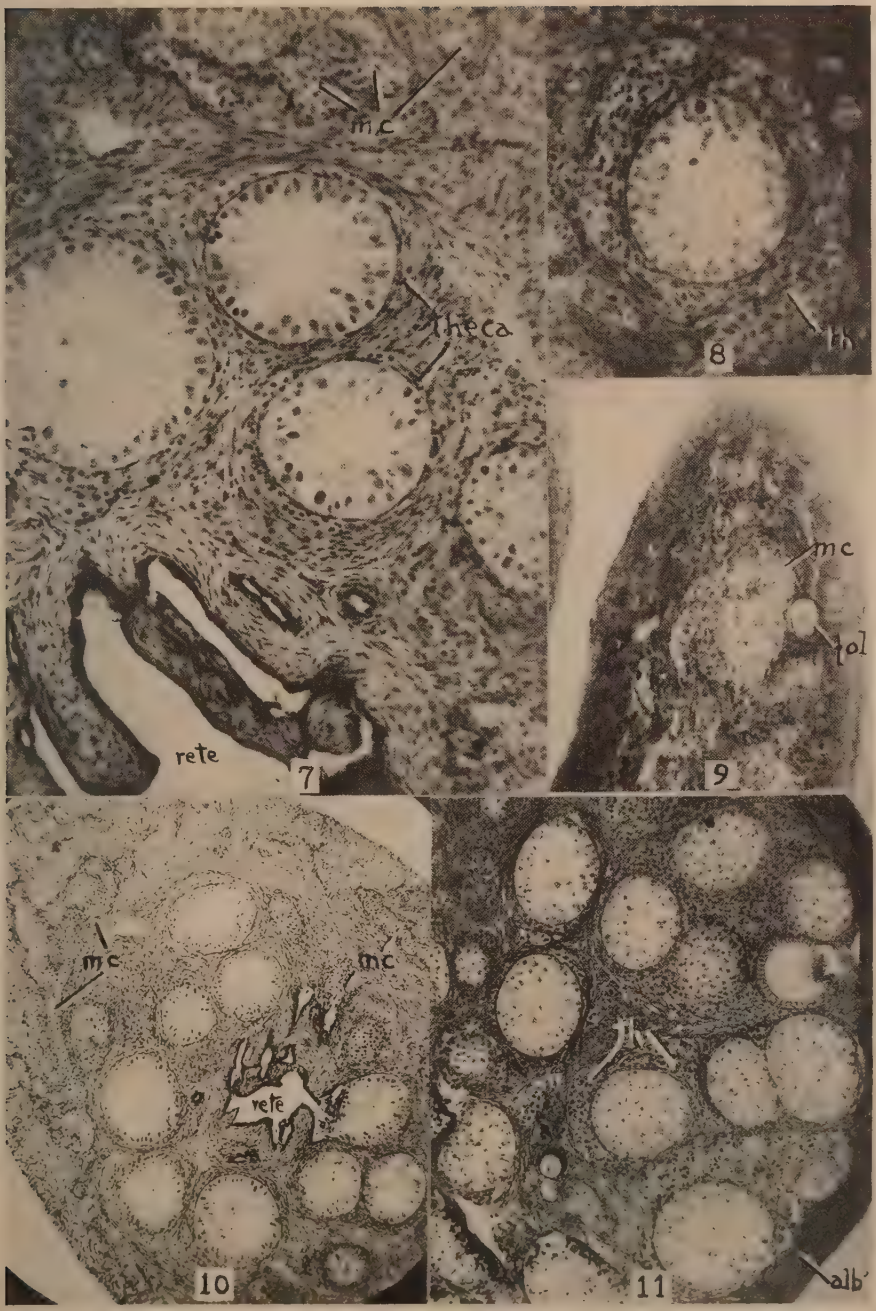
7 Portion of section through gonad of the sex intergrade, showing rete ovarii (*rete*), anovular follicles with thecae, and medullary cords (*mc*). × 200.

8 Anovular follicle from ovary of adult animal no. 593. *Th*, theca. × 200.

9 Process on adult ovary of no. 307, showing medullary cords (*mc*) and one Graafian follicle. × 100.

10 Greater portion of section through gonad of sex intergrade, showing relation of rete ovarii, anovular follicles, and medullary cords. × 50.

11 Numerous anovular follicles in an adult ovary no. 593. *Th*, theca; *alb.*, albuginea. × 100.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THE REFRACTIVE GRANULE RED BLOOD CORPUSCLE. ITS BEHAVIOR AND SIGNIFICANCE[1]

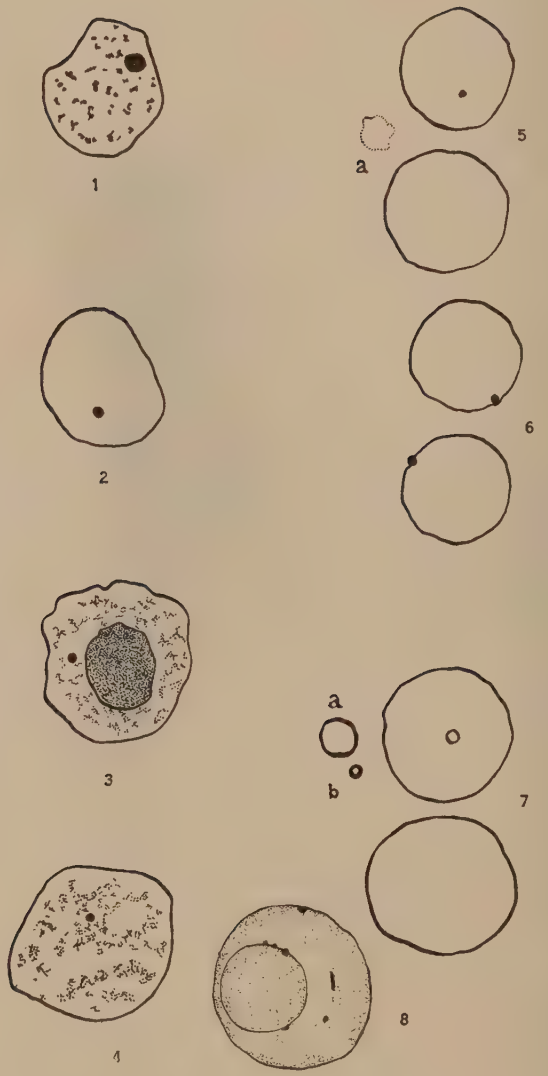
RAPHAEL ISAACS
Boston

FIFTEEN FIGURES

The recognition of reticulation in supravitally stained red corpuscles as an evidence of youth, by Cesaris-Demel(1) and by Meves(2), has served to explain many problems in red-cell behavior. Nucleated and reticulated red cells have been considered the two main types of normal inmature red cells. Recent experiments(3) have shown that these two types of red blood cells have certain properties in common in that they do not agglutinate readily with normal agglutinating sera which readily clump the adult cells and they do not hemolyze as readily as the adult cells with hemolytic sera. There is a group of red cells showing as their only internally differentiated structure a refractive granule, which have the same properties as the immature red cells with these agents. This paper deals with the cytological aspects of these refractive granule red cells. The physiological and clinical features have been previously recorded(3, 4, 5).

While studying the residue after serum hemolysis of red blood corpuscles of man and of the dog, certain non-hemolyzed red cells were encountered which contained a highly refractive granule. Similar granules were recognized in fresh blood mounted under cover-glasses sealed with petrolatum. Granules of the same type were then identified

[1] From the Medical Service of the Collis P. Huntington Memorial Hospital of Harvard University. Part of the expense of this investigation has been defrayed by the Edward Hickling Bradford Fellowship.



Figures showing granule red cells, with normal cells and Howell-Jolly body cell for comparison.

Fig. 1 Reticulated red cell, showing Howell-Jolly body. (Secondary anaemia–malignant lymphoma.) This and the other figures are camera-lucida drawings

in the red cells of stained films of normal and pathological blood. The development of the granule was traced back through supravitally stained reticulated and nucleated red cells, and quantitative studies were made(3, 4, 5). Granule red cells have been identified, so far, in the blood of man, dog, rabbit, and mouse.

THE GRANULE: APPEARANCE IN FRESH AND STAINED ERYTHROCYTES

The granules, one to a cell, appearing in about 1 per cent or less of the red cells in healthy individuals, measure 0.5 μ in diameter. They appear either centrally or peripherally located (figs. 5, 6, 9) and in some cells can be seen on the edge of the disc (figs. 6, 9). In nucleated red cells the granules can be seen in supravitally stained (brilliant cresyl blue) preparations as a refractive globule near the nucleus, surrounded by or adjacent to a clear area (fig. 8). This can sometimes be recognized in stained films (Wright's stain) (fig. 3). In reticulated cells mounted in a moist preparation and supravitally stained with brilliant cresyl blue the granule stands out unstained and highly refractive, either in the meshes of the reticulum or well separated from it (fig. 4).

at table level. Zeiss ocular 12, objective 2 mm. (oil immersion). Cells supravitally stained with brilliant cresyl blue and counterstained with Wright's stain.

Fig. 2 Red cell, showing refractive granule (same blood).

Fig. 3 Nucleated red cell, showing reticulum and refractive granule (same blood).

Fig. 4 Reticulated red cell, showing refractive granule (same blood).

Fig. 5 Normal blood (stained as above), showing refractive granule red blood cell (above) and mature red blood corpuscle (below). A blood platelet (*a*) shown for comparison.

Fig. 6 Two refractive granule red cells (normal blood), showing granule at periphery.

Fig. 7 Normal blood (fresh) mounted under a cover-glass rimmed with petrolatum. No stain. Refractive granule red cell (above) and mature red corpuscle (below). At (*a*) is shown a blood platelet, and at (*b*) a refractive granule, free in the plasma.

Fig. 8 Nucleated red blood corpuscle. Supravitally stained with brilliant cresyl blue and mounted in the fresh state under a cover-glass sealed with petrolatum. (Chronic myelogenous leukemia.) The refractive granule is seen touching the right lower edge of the nucleus.

It is less easily recognized in the polychromatophilic and basophilic cells in films colored with Wright's stain, either alone or superimposed on a supravitally stained preparation, because the differences of refraction are not so marked. In such preparations the granule red cells, which show no other structure besides the granule, are easily identified (figs. 5,

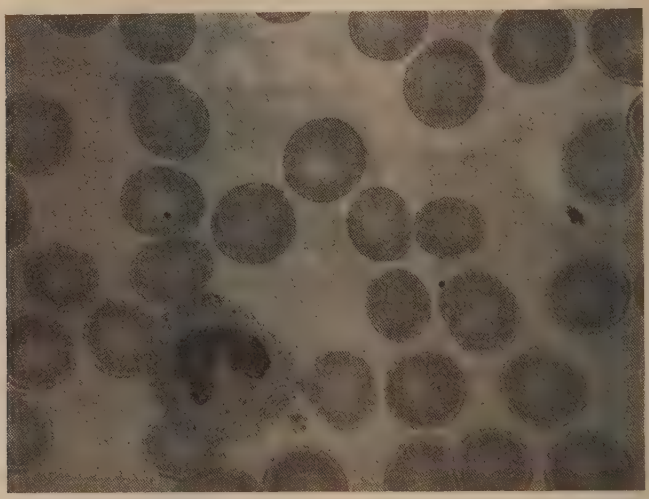


Fig. 9 Photomicrograph of a film of blood from a normal, healthy man (brilliant cresyl blue, Wright's stain). In the left side of the field is a red cell containing a refractive granule. On the right side is shown a cell with a granule on the edge. The comparative size of a blood platelet (right) is shown. The granule in the reticulated cell (right, upper) is barely visible, but the different character of the reticulum stain is shown.

9). In these the granule appears a clear, black dot when the cell is in focus and a brilliant refractive disc when the cell is just out of focus (figs. 9, 10). These cells stain the usual red with eosin. The granule does not take any of the ordinary stains, but sometimes appears colored because of the surrounding stain. With brilliant cresyl blue the granules sometimes appear pink in wet preparations—a reflex color characteristic of this stain.

The granules, in the fresh preparations, appear to be free within the cells and exhibit some motility, probably of the nature of Brownian movement. They have been identified free in the plasma, where their dancing movement is marked. When pressure is exerted on the cells, the granule can be extruded as an intact globule which retains its identity. It resists heating to 60°C. for at least thirty minutes, and apparently is not much changed when heated to a somewhat

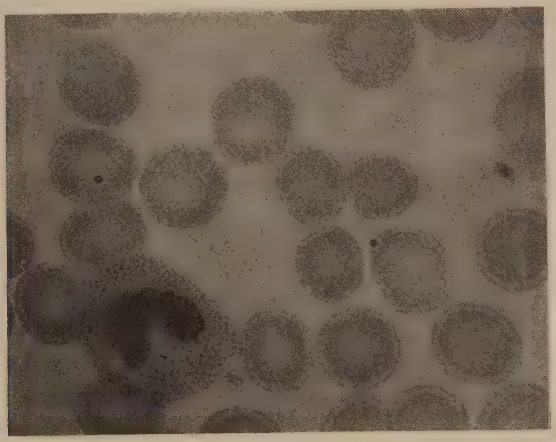


Fig. 10 The same field as in figure 8, but slightly out of focus, to show the refractive nature of the granules.

higher temperature or for longer intervals. So far, its chemical nature has not been determined.

The red blood corpuscles containing the granule as the only visible structure have certain differentiating characteristics. They resist hemolysis with hypotonic salt solutions and with hemolytic sera more than the completely mature corpuscles, and are among the last to lose their hemoglobin. Cells with these granules also appear to be more resistant when blood is heated to 55° to 60°C. for thirty minutes than the fully developed red corpuscles. These granule red cells also do not agglutinate with agglutinating sera.

DIFFERENCE FROM HOWELL BODIES AND NUCLEAR PARTICLES

The granules described in this paper do not take the nuclear stains as do the Howell bodies(6) or nuclear particles (Jolly, 7). When Wright's stain is used, the nuclear particles are bluish, the Howell bodies very deep blue, and the granules black. The granule is just as brilliantly black in cells stained only with eosin as in those stained with eosin-methylene blue combinations. Unlike the Howell bodies and nuclear particles, the granules do not stain with methyl green or with brilliant cresyl blue. Unlike nuclear particles, the granules

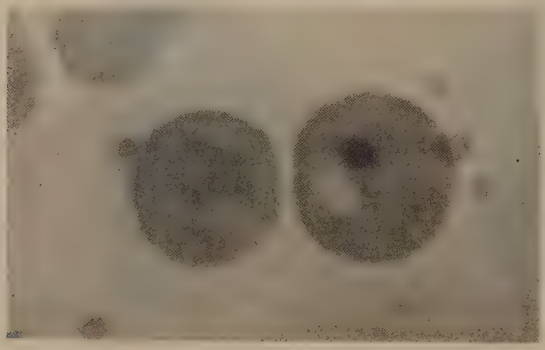


Fig. 11 Howell-Jolly body in red blood cell. (Secondary anaemia. Post-splenectomy. Brilliant cresyl blue supravitally. Wright's stain.)

do not 'disappear' from unstained blood films kept for months (Morris, 8). The Howell bodies and nuclear particles appear less refractive in stained films, and when compared with the granules, the duller appearance is quite evident (figs. 11, 12). The granules are smaller than the average Howell body which appears so frequently in the blood after splenectomy (fig. 12). Some of the nuclear particles are of the same size as the granule, but the granule with which this work is concerned is always single, while the nuclear particles are occasionally multiple(8). The granules are constantly present in normal blood in healthy individuals where Howell bodies and nuclear particles are never encountered. Some

of the structures which have been pictured erroneously in the literature as Howell bodies are undoubtedly the refractive granules.

DEVELOPMENT

In practically all of the nucleated and reticulated red corpuscles examined with brilliant cresyl blue in wet preparations, it has been possible to locate the granule. This is more

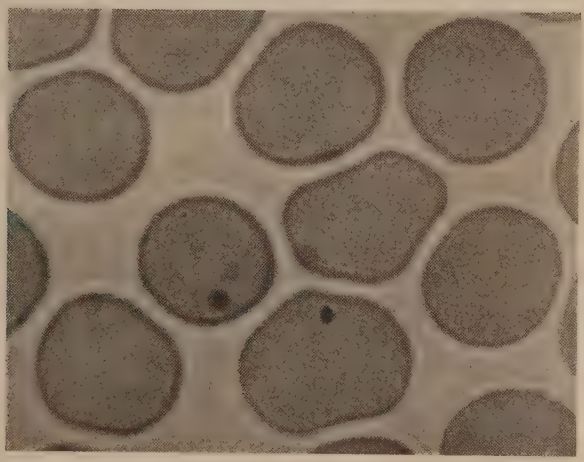


Fig. 12 Howell-Jolly body (left) and refractive granule (right) in red blood cells. (Secondary anaemia. Postsplenectomy. Brilliant cresyl blue supravitally, Wright's stain.) The photomicrograph shows the brilliant refractive nature of the granule in comparison with the duller appearance of the Howell-Jolly body. The former appears deep black, the latter deep purplish blue. The granule has been frequently confused in the literature with the Howell-Jolly body and classified as such.

difficult in stained films, but it is frequently possible to note its presence, although its position, the depth of surrounding stain, and the lack of contrast often hide it or reduce its visibility. The evidence from the fresh moist preparations makes it seem quite possible that the granules are present throughout the nucleated and reticulated stage, and in the next stage in which the red cells appear otherwise mature.

The appearance of a granule on the edge, but tightly adherent to some corpuscles, and of others at times free in the plasma suggests that in the course of development there is a persistence of this structure through the stages of immaturity and that *the cells showing only the granule represent a very late stage in the maturation of the red blood cell.* In the bone-marrow the percentage of cells showing the granule is high, and in preparations studied (dog) 40 per cent of the red cells which did not show a nucleus or reticulum showed the granule. The order of appearance of young red blood cells following hemorrhage, transfusion, and irradiation, given below, further substantiates the view that the granule cell is a late stage in the maturation of the red cell.

OCCURRENCE AND DISTRIBUTION

In 175 adults in apparent health the number of granule red cells varied from 0.3 per cent to 0.7 per cent in the majority while at rest and seldom was over 1 per cent. Counts of 3.0 per cent or over usually indicated some abnormal condition. The maximum number encountered so far in adults is 8.9 per cent (carcinoma of cervix with hemorrhage under treatment with short-wave Roentgen-ray irradiation). In a two-day-old mouse 13 per cent were present; 15 per cent have been counted after hemorrhage in dogs. The cells were enumerated in cover-glass films supravitally stained with brilliant cresyl blue, and counterstained by Wright's stain, and from 1000 to 10,000 cells were counted, according to the uniformity of the preparation.

Exercise appears to cause a rapid temporary increase (1 to 3 per cent) in untrained individuals within an hour, but the routine of a day's work without violent exertion causes a variation of within 1 per cent. Chart A shows the variations in the reticulated and granule red-cell count in a normal individual from hour to hour. It will be noted that there appears to be reciprocal relation between the two and that their sum remained fairly constant throughout the day. In trained athletes at the height of their training the number

was found to be higher than normal, the average of seventeen Marathon runners being 3 per cent, with a maximum of 6.6 per cent (extremes 1.4 to 6.6 per cent). In contrast to the effect of exercise in untrained persons, these athletes showed no great change in the percentage of granule red cells after running a twenty-six-mile race.

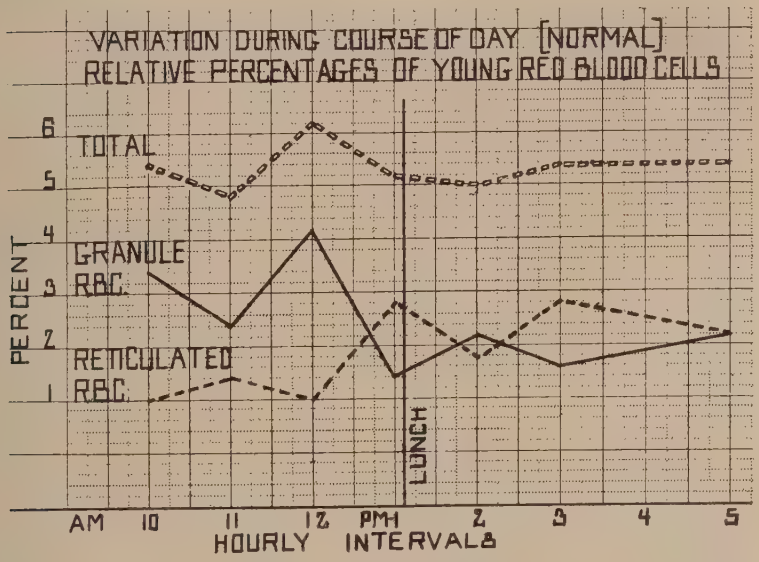


Chart A Percentage variation of reticulocytes and granule red corpuscles in health during the course of a day. (Man.) The reciprocal relationship between the two stages is shown.

After an acute hemorrhage there is a rapid rise in the number of granule red blood cells in the peripheral circulation, starting on the day of the hemorrhage and reaching its maximum eighteen to twenty-four hours later. If the loss of blood is acute and small (e.g., less than 500 cc., as from a donor for transfusion), the increase in the numbers of granule red cells may be the only change that the red blood corpuscles show. If the loss of blood occurs in an otherwise normal individual and is great, the reticulated cells begin to increase

in the peripheral circulation usually on the second day (chart B) and increase rapidly, while the relative number of granule red cells decrease. With restoration of the blood, the relative numbers of reticulated cells and granule cells return to their original level. A second hemorrhage occurring before the return to normal gives a somewhat similar response on the part of the red blood corpuscles, but the fall of the relative number of granule cells is likely to be more precipitate and the rise of the number of reticulated cells more rapid, especially if the hemorrhages occur in close succession.

As noted by Vogel and McCurdy(9), Robertson(10), and Krumbhaar and Chanutin(11), after transfusion of compatible blood in man and in dogs, the relative number of reticulated cells first decreases in the peripheral circulation on the day of the transfusion and on the following day (first twenty-four to thirty-six hours), then rapidly increases during the next three or four days (chart C), sometimes longer. This takes place whether blood loss has preceded the transfusion or not(3, p. 203). The granule red corpuscles do just the reverse. During the course of the first twenty-four to thirty-six hours after the transfusion, there is a rapid relative increase in the per cent of granule red cells, followed by a gradual decrease during the following days. When the production of new red blood corpuscles is sufficient to fulfill the needs of the circulation, the number of granule cells and reticulated red cells decrease in number until they reach their normal level(3). This may not occur for from ten days to over three months, according to the nature of the disease of the patient, or the insufficiency of red corpuscles may continue until death. After transfusions which are not effective in aiding blood formation, the rise in number of young cells is insignificant or absent. An effective rise, heralding a desirable response of the blood-forming tissues, may follow the introduction of a very small quantity of blood, and in those cases which do not require replacement of a large quantity of fluid, a transfusion of from 300 to 500 cc.

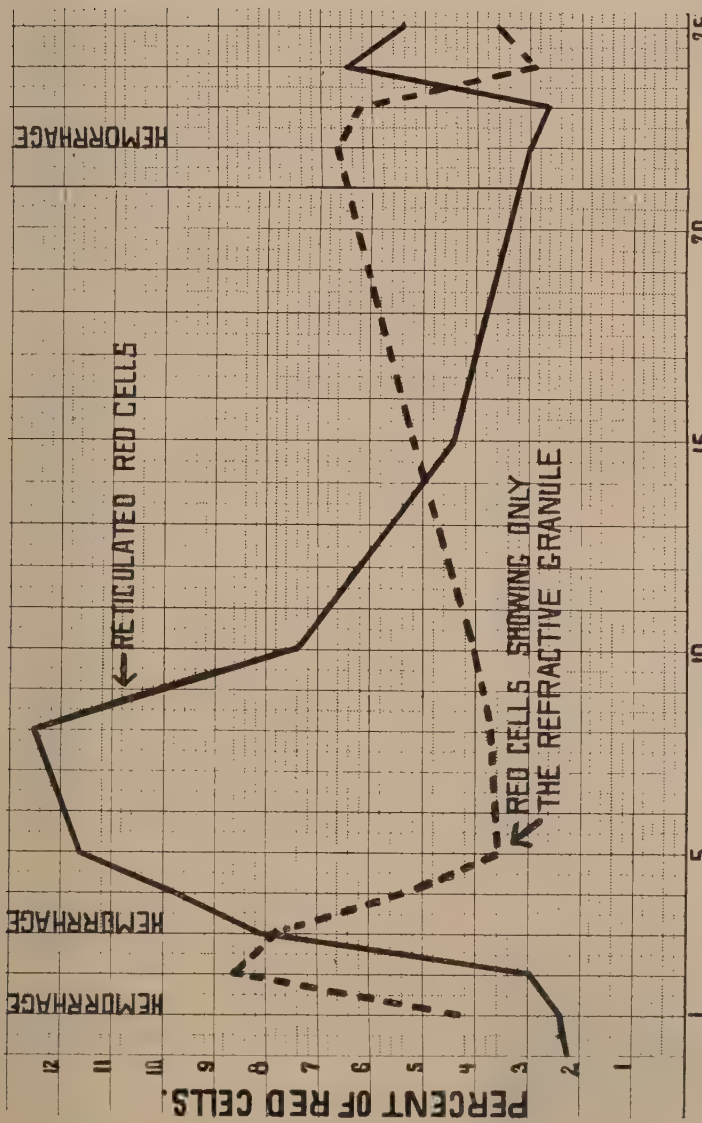


Chart B Effect of repeated hemorrhage on young red-cell production. (Dog.) The reciprocal relationship of the percentage of reticulocytes and granule red corpuscles is shown. The hemorrhages represented 170, 100, and 200 cc., respectively, in a dog weighing 7 kilo.

of blood appears to be ample for the purpose of marrow stimulation. No gross difference appears in the response of the young cells after citrated or after whole blood transfusions.

Intensive short-wave Roentgen-ray irradiation over any part of the body is followed within a few hours by a decrease in the percentage number of reticulated cells and a rapid increase in the percentage number of granule cells in the

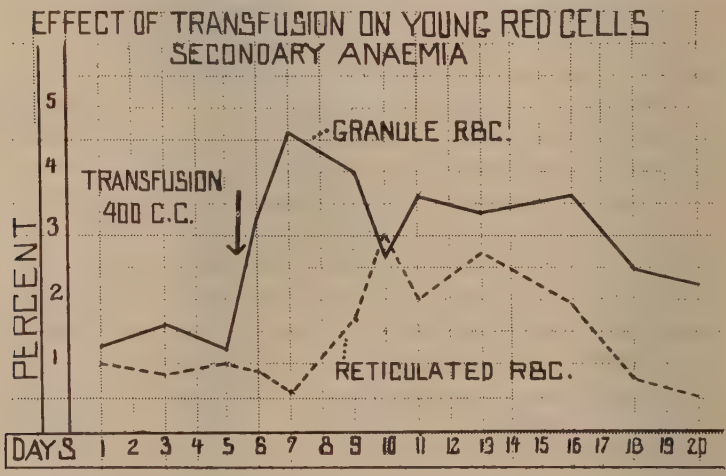


Chart C Effect of transfusion on the young red cells (secondary anaemia, Hodgkin's disease). The depression of the percentage of reticulocytes and increased production of granule red corpuscles just after the transfusion and their subsequent reciprocal relationship is shown. Finally, both kinds decrease in number and approach the normal rate of production.

peripheral circulation. This effect lasts for varying lengths of time, from a few hours to several days, depending on the precise dosage and the state of the given patient. The detailed data are in process of publication.

The characteristics of the quantitative behavior of the young red corpuscles in any disease or following transfusion or irradiation are not typical or diagnostic of the disease, but merely represent the rate of blood maturation at the given time.

PHYSIOLOGICAL AND PATHOLOGICAL SIGNIFICANCE

Clinical observations seem to enhance the view that the refractive granule containing red corpuscle represents the final stage of the maturation of the erythrocyte. The exact place where this stage normally is completed, in the bone-marrow or in the peripheral circulation or elsewhere, has not been determined. The recognition of this stage throws further light on the response of the blood-forming tissue to various stimuli. Thus, while the temporary reduction in the per cent of reticulated red blood cells in the peripheral circulation below normal after transfusion often has been interpreted as indicating a 'depression' of the bone-marrow, it can be considered as indicative of a 'stimulation' of the bone-marrow to send out the corpuscles in the next mature stage—that of the granule red cell. This view also helps to interpret the latent period in the appearance of an increased number of reticulated red cells in the peripheral circulation after hemorrhage—namely, it is the time when the cells come out of the marrow in the next stage of maturity. The granule red cells are delivered until the demand is so great that the more immature cells must enter the blood stream. The recognition of this cell helps, also, in understanding the so-called aplastic anaemias, where cells are being produced, but very few reticulated cells are discovered. The level of production here is very low, but the cells reach an advanced stage of maturity. This is a contrast to the usual condition in pernicious anaemia, where the majority of the red cells in the marrow never reach maturity, just as in the typical malignant conditions (carcinoma, sarcoma), where the tumor cells go as far as the multiplication stage, but do not mature much beyond this.

The recognition of this additional stage in the maturation of the red cell offers an opportunity for the further study of changes in the rate of red-cell production and destruction in health and disease and for the study of the changes produced after therapy, both experimental and clinical.[2]

[2] The photomicrographs appearing in this paper were made by Mr. Ernest E. Fewkes, of the Department of Anatomy of Harvard Medical School.

SUMMARY AND CONCLUSIONS

The final stage in the maturation of a red blood corpuscle in the blood of man, dog, rabbit, and mouse is recognized by the presence of a single, brilliant refractive granule (black, in stained blood films), $\frac{1}{2}$ μ in diameter. In health and while at rest about 0.3 to 0.7 per cent of the red cells in the peripheral circulation (man) are in this stage. Under average physiological conditions, the number varies around 1 per cent. When an increased production of red cells is stimulated, these cells are the first to reach the peripheral circulation after the rate of production of mature red cells is insufficient. When the requirements cannot be met by the delivery of adult and granule red cells, the reticulated cells increase in the peripheral circulation, and if there is still further demand, and the bone-marrow is not pathological, the nucleated form appears in increased numbers. The recognition of this stage in the development of the red blood corpuscle has thrown further light on the behavior of the erythrocytes after, *a*) exercise, *b*) hemorrhage, *c*) transfusion, and, *d*) Roentgen-ray irradiation.

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AN IMPROVED LABORATORY TABLE FOR GROSS ANATOMY

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ONE FIGURE

The table to be described was designed by the author in the year 1912–13 and was made and introduced into the dissecting laboratories of the new School of Medicine, Washington University, in the spring of 1914. It has been in use by medical students and other students of anatomy and anthropology for the past ten years; therefore sufficient time has elapsed in which to have tested this new type of laboratory apparatus.

The Wolff Plumbing Company of Chicago built and installed the table. It consists of a steel framework providing lockers and supporting a stone top with a sink and water connections; an electric plug is mounted on the frame under the table top. This type of table was designed for two dissectors, but it is serviceable for a larger group.

The dimensions are: length of top, 203 cm.; breadth, 61 cm.; height of upper surface from the floor, 80 cm. The base measures 189 cm. long, 49 cm. wide, and is overhung by the top 6 cm. at the sides and 7 cm. at the ends. It will be noticed that this table is lower than the usual form. I regard the height of the dissecting table which prevails as unnecessary and awkward for the worker. With the cadaver resting on blocks on the top of the present table the working level requires a seat level about 10 cm. higher than that of an ordinary chair. When the table is used for small dissections, drawing, writing, etc., the seat of the revolving stool occupied by the worker is at the level of that of an ordinary chair.

Two square sheet-metal pillars, one at each end of the table, make the supports for the heavy top. They give space for the three lockers and house the plumbing. In one of the pillars (at the right in the photograph) are two individual lockers opening on opposite sides of the table, each with two shelves for the student's instruments and books. In a narrow closet between the backs of these lockers the plumbing runs from the sink straight to the floor. In the second pillar is a common locker for the use of both students; it contains the book rests, lamp support, blocks, oil-skins, and other pieces of equipment for the table when not in use. The common locker is used also for storing jars of material in the intervals between the periods of study.

The table-top rests upon a steel frame and is level, not sloping; it is made of one piece of alborene, a hard soapstone, strong and resistant to corrosive agencies. Drainage of the fluids from the body is managed by grooves in the top, beginning shallow at one end of the table and gradually deepening as they approach the sink at the opposite end where they discharge. The sink measures 33 cm. $\times$ 36 cm. and is 20.5 cm. deep. It is provided with a water-tap and drain. This sink functions in the technical work, such as emptying hollow viscera, washing out blood clots, cleaning instruments, etc.

The forged-steel frame supports a projecting rail on each side running the full length of the table just beneath the edge of the top. To this rail are adjusted the book rests and lamp support, which may be moved to any desired place along the table and made secure by set-screws. The rail was intended as a support for apparatus of all sorts needed in gross anatomy and physical anthropology: it will accommodate a binocular microscope, measuring and drawing instruments, a photographic camera, clamps and supports for holding dissections, etc.

Artificial lighting of the dissection is effected by means of a combination of the overhead general lighting of the laboratory and a table lamp. By this arrangement shadows are almost entirely avoided. The table lamp, an ordinary

60-watt bulb covered by a bell-shaped metal shade, is suspended from a special support. This is a metal rod formed like an inverted L, the vertical limb slipping into a metal tube which in turn slips into a bronze casting formed to grip the rail described above. The lamp, hanging on the horizontal arm, may be moved its length in directing the light over the dissection, and it may be raised and lowered

Figure 1

to the level desired by means of the sliding vertical rod and tube. The parts of the support can be made stationary and firm by thumb-screws. Electrical connection is brought about by a cord and plug fitting the socket under the table. The current is of course available for power.

The problem of a convenient book rest was solved also by the adaptability of the steel rail. In this case a vertical telescoping rod and tube set in the bronze casting forms a

support surmounted by a sheet-metal book rest. The support is adjustable for height and for position along the table and can be rotated on a vertical axis.

In the laboratory the tables are placed in rows of two or three, end to end without intervening spaces; the distance between rows is 6 feet, giving enough room for workers at the tables, passageway between them, and space for all the operations of the technicians and janitors.

The mechanical advantages of this type of table over the old kind and the many possible uses are apparent. Of no small consequence is the training gotten by the student in handling and caring for the apparatus, simple as it is, which must in the course of the year produce good effects. The initial cost of the table is high in comparison with that of the usual type, but its advantages are worth the difference; the expense of maintenance is very slight.

SOME LABORATORY APPARATUS AND METHODS FOR EMBRYOLOGICAL AND CYTOLOGICAL WORK

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FIFTEEN FIGURES

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A METHOD OF RIPENING HAEMATOXYLIN

Every cytologist and histologist is familiar with the need of allowing haematoxylin stains to stand several weeks or months to become oxidized or 'ripened,' and with the use of certain oxidizing agents, such as hydrogen peroxide and potassium permanganate, for hastening the process. Although haematin, an oxidation product of haematoxylin, may be used in place of haematoxylin for making up a stain that acts at once, it seems to be the common practice to use haematoxylin and to wait for spontaneous ripening.

In order to avoid the long period of waiting and also the addition of oxidizing reagents, the writer experimented some years ago with other methods of ripening haematoxylin solutions. Advantage was finally taken of the well-known oxidizing properties of platinum black, which when added

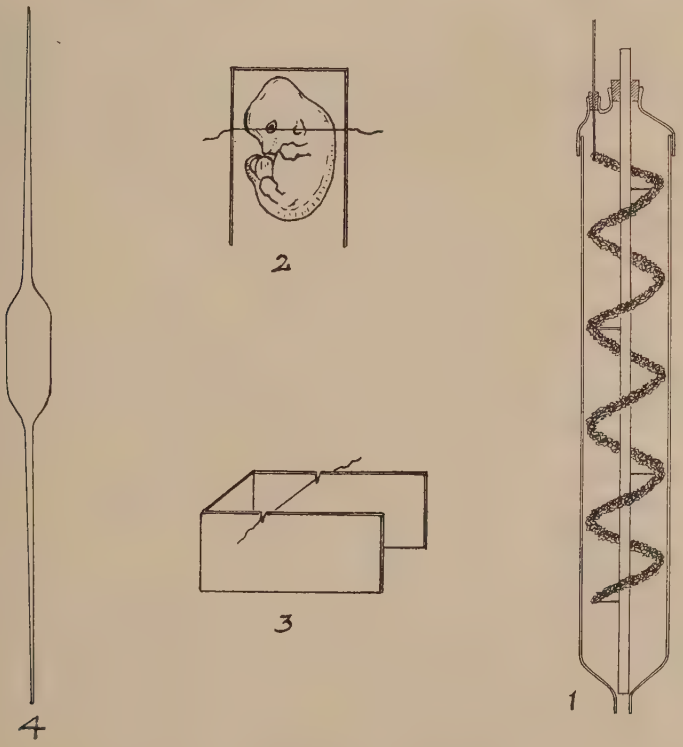
to a solution of the dye immediately ripens it. Platinum black in the form of a loose powder is, however, open to the objection that it is not easily recovered without loss from a solution. To avoid this objection and yet to utilize in a simple form the peculiar properties of platinum the following apparatus (fig. 1) was devised in which the platinum black was caused to adhere to asbestos wool, which in turn was supported on a platinum wire.

For the sake of compactness and ease of manipulation, the piece of platinum wire, about 70 cm. long, first bent into the form of a spiral, was supported about a glass rod. Pure asbestos wool saturated with a solution of platinum chloride was then plastered bit by bit over the wire so as to cover it and form a rough, fuzzy, continuous mass; the whole being finally roasted in a gas furnace out of contact with the flame until the chloride was decomposed and the platinum reduced. The platinum black thus formed rendered the asbestos slightly stiffer and firmer—a condition which is scarcely changed when the asbestos is subsequently saturated with water. The spiral is enclosed in a glass cylinder both for protection and to prevent undue evaporation of the solution which is being ripened.

It will probably be evident that a solution of haematoxylin may be ripened merely by allowing it to trickle over and through the spiral. The aqueous solution, for example of $\frac{1}{2}$ per cent, of a light rose color when neutral, enters at the top, moistens the spiral in its passage downward coming into contact with the platinum black, and drops from the lower end a rich, deep crimson fluid perceptibly more viscous as indicated by the manner in which it adheres to glass. The degree of oxidation depends in part on the rapidity of flow through the spiral, and this rapidity in turn is largely determined by the rate at which the solution is allowed to enter the spiral. The ripening action is best on a simple, aqueous solution of haematoxylin, and apparently does not occur in a mixture like phosphotungstic acid-haematoxylin. The ripened aqueous solution may, however, be mixed with the

desired ingredients and be ready at once for staining tissues. It may be preserved indefinitely without further spontaneous oxidation if kept in full, tightly stopped bottles.

After one lot of solution is ripened, the spiral may be washed by running considerable quantities of hot distilled



Figures 1 to 4

water over it. It seems to work repeatedly and continuously, the last of a solution being changed to practically as deep a color as the first. A good rate at which to allow the fluid to run is about 25 cc. per hour.

The rate of flow is best controlled by means of a capillary siphon. In fact, the capillary siphon, easily made by heating

and rapidly drawing out a piece of glass tubing, is without doubt the best means for obtaining a regular, uninterrupted flow of any fluid. Any rate of flow may be had by varying any or all of the following three conditions: 1) difference in height between the outlet and inlet, 2) size of bore, and, 3) total length of the siphon, for obviously the longer the tube the greater the internal friction, and consequently the slower the flow. With a long tube of comparatively large bore there is the least possibility of particles in the fluid obstructing the lumen. The capacity of a siphon is easily calculated by allowing a solution to run through it into a graduated pipette laid on its side and can be made as desired after a few trials.

A METHOD OF MARKING THE ORIENTATION OF OBJECTS EMBEDDED IN PARAFFIN

Control of the exact plane of sectioning is sometimes a matter of considerable importance, especially in the study of embryos and in the reconstructing of them in wax, and may be obtained by the following method.

A frame of sheet aluminum thick enough to be fairly stiff is made in the form shown in figure 3. It should be large enough so that 2 to 4 mm. of space may be left between it and the object about which it is to be placed. As indicated in figure 3 a very fine black silk thread or a strand of fine thread is held taut by being forced into two notches made with a sharp scalpel. The position of the thread may of course be varied with the needle. The frame with its thread in position should be in readiness in a dish of melted paraffin. Air may be prevented from being entangled in the thread by first moistening it with xylol.

The object to be embedded, for example a small embryo, is first placed in such a position, with the aid of a binocular microscope, that the desired plane of sectioning shall be vertical, and, if the object is being embedded in a vessel with a flat bottom, perpendicular to the bottom. Syracuse watch-glasses are excellent for this purpose. The object

is easily made to retain the desired position if it is transferred to the embedding dish after the bottom of the dish has been slightly cooled or just enough to congeal a thin layer of paraffin. Next, while the paraffin above and around the object is still fluid and clear, the aluminum frame with its thread is quickly placed in position as shown in figure 2. If, beforehand, the plane of sectioning is marked on a photograph or sketch, it is only necessary to adjust the frame so that the thread has a corresponding relation to the object. This can be done rapidly and with great precision under a binocular, especially if the thread lies only 2 or 3 mm. above the object.

After the paraffin is hardened the frame will be found to be easily separated from the paraffin, leaving the thread embedded. If the frame is small enough, very little trimming is necessary. The paraffin above the object should be removed down to the thread. The block may now be oriented in the microtome with the original undersurface horizontal and the thread parallel to the knife edge. After orientation is complete, enough more of paraffin may be trimmed off to remove the thread. Or, if the thread was placed in the frame parallel to the end of the frame and the frame is placed with its end perpendicular, the orientation in the microtome may be made with regard to the end surface of the block.

ON KEEPING OSMIC-ACID SOLUTIONS AND MIXTURES

Various methods for preventing the reduction of osmic acid are described, such as adding chromic acid, potassium permanganate, or corrosive sublimate to the solution. While these may be effective, they are not necessary, since all that is required is to make up the solution in double distilled water and to use vessels and containers rendered scrupulously clean. It is important also to prevent loss by volatilization and evaporation.

To attain these desired ends it has been the custom of the writer to keep both 2 per cent solutions of osmic acid and also osmic fixing fluids, such as Flemming's and Benda's mixtures, sealed in glass bombs. The latter are made from

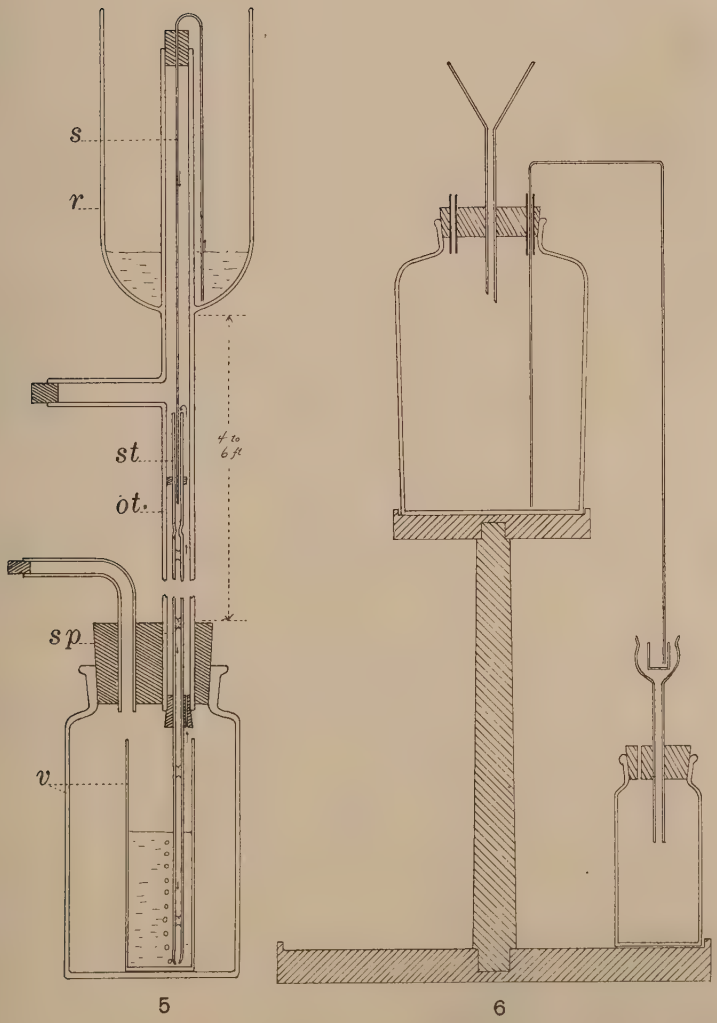
pieces of glass tubing having a diameter of about 22 mm. The tubing is first carefully cleaned and then drawn into the form shown in figure 4, with slender tapered ends 15 to 30 cm. long, and of the desired capacity. The bombs are easily filled by exhausting the air through a wash-bottle, the ends then being sealed in a flame. To use a part of the contents the tips of the two ends are cut off, the desired amount poured out, and the ends again sealed.

Osmic-acid solutions and mixtures can thus be kept indefinitely with the assurance that they are not diminished in value by evaporation or by escape of the osmium vapor.

APPARATUS FOR THE GRADUAL MIXING OF FLUIDS

On starting to set up an apparatus described by Ezra Allen ('16) for the gradual dehydration of tissues, certain modifications suggested themselves, which have been incorporated in a new piece of apparatus and used successfully. The excellent method of blowing air through the fluid to which alcohol or other reagent is being added in order to insure constant mixing is adapted from Allen's apparatus, but the method of obtaining the air supply and of controlling the flow of fluid, as well as the general arrangement, is new.

It will be seen from the sectional drawing of the apparatus (fig. 5) that the four essential parts are: 1) an upper reservoir (*r*) from which the fluid to be added is withdrawn through, 2) a capillary siphon (*s*) which extends a short distance into, 3) a long piece of small tubing (*st*) having a bore 1.5 to 2 mm. in diameter which receives the fluid from the siphon and carries it to the bottom of, 4) a vessel (*v*) which contains the tissue in the fluid to be changed. This vessel may be a smaller vial set inside a larger one if desired, as shown in the figure. Near the upper end of the small tubing (*st*) and below the end of the siphon is a slight constriction which causes the fluid delivered by the siphon to form drops which fill the lumen. These drops are formed at intervals, and as they fall they draw in air at the upper end of the small tube and carry it to the bottom of the vessel (*v*) where



Figures 5 and 6

it is liberated to rise as bubbles through the fluid which it thus keeps in circulation. The fluid added, when lighter, as in the case of alcohol to water, or xylol to alcohol, is here introduced at the most advantageous level to facilitate mixing. Thus the descending fluid causes itself to be mixed thoroughly with that in the receiving vessel (*v*). The rate at which it flows is determined by the dimensions of the siphon. The length of the spaces between drops, and consequently the number of drops in the tube at any one time, depends on the length of the small tubing and on the depth of fluid through which air must be forced. That is, the sum of the lengths of all the drops, making allowance for friction, must be a little greater than the depth of the fluid being changed in the vessel (*v*). In order to get this necessary pressure for ordinary purposes, the tube (*st*) should be about 5 feet long. The volume of air blown through the fluid will vary somewhat between ten and forty times the volume of fluid delivered by the siphon; in any case, being amply sufficient to mix the fluids so thoroughly that stratification can never be detected. To facilitate the use of the apparatus and for protection to the siphon and small tubing, the latter are encased in a larger outer tubing (*ot*) which is blown in one piece with the upper reservoir (*r*) and at its lower end passes through the stopper (*sp*) of the lower vessel (*v*). It supports the siphon and inner small tubing as shown in figure 5. The whole apparatus, which is about 6 feet long, is most conveniently hung from a rack, the upper reservoir serving to support the whole apparatus so that the lower part is at table level and easily accessible. The rack may resemble those often used to hold and drain large graduate glasses, and consists of a flat board suitably supported, into the front edge of which a deep cleft is cut wide enough to receive the outer tube, and rounded and beveled at the inner end for the bottom of the reservoir. Another advantage of the outer casing lies in the fact that it forms a passage for the return of air already blown through the fluid to the upper end of the small tubing. Thus the same air is used over and over,

making unnecessary any apparatus for drying it, and also reducing evaporation to a minimum. The decrease in the space occupied by the air resulting from the accumulation of fluid in the lower vessel does not lead to increased pressure in the enclosed air because some can escape around the stopper pierced by the siphon. The leakage, however, is not so great as to interfere with starting the siphon by partially exhausting the air through either the side neck in the casing or through a tube in the stopper of the lower vessel.

The siphon runs until all the fluid in the upper reservoir is exhausted. It is a simple matter to calculate the amount of fluid, alcohol, for example, of known strength that should be put into the reservoir in order to reach a desired concentration in the lower vessel which contains a known quantity and strength at the beginning. The following table will be found sufficiently accurate for practical purposes. No allowance is made for fluid in tissues.

It will be seen that with the same siphon the rate of dehydration will depend on the strength of alcohol used and also on the volumes employed. Thus, with a siphon delivering 4 cc. per hour, a small piece of tissue in water can be got into 71 per cent in from three to twelve or more hours if

FLUID TO BE CHANGED. 1 VOLUME OF	STRENGTHS OF ALCOHOLS TO BE ADDED AND VOLUMES IN ORDER TO OBTAIN PER CENT INDICATED UNDER 'RESULT'									
	30 per cent		50 per cent.		70 per cent		95 per cent		100 per cent	
	Volumes	Result	Volumes	Result	Volumes	Result	Volumes	Result	Volumes	Result
		Per cent		*Per cent*		*Per cent*		*Per cent*		
H_2O	2	15			2	35	3	71		
							5.5	80		
15 per cent			.8	30+						
30 per cent					1.2	50+				
50 per cent							1	72+		
71 per cent							1.78+	80	4.5	95
80 per cent							3	91	3	95
91 per cent									3	98—
95 per cent									3	99—

95 per cent is added. It is possible also to extend the whole process over several days in the case of very delicate structures. An intermittent siphon as used by Allen may also be attached to v, thus making steps indicated in the table practically continuous.

The apparatus can be used for simply keeping a solution agitated by filling the reservoir with fluid like that in the lower vessel.

It can be made as large as need be, and, if desired, two or more can be arranged to introduce alcohol and air at different parts of a large container.

APPARATUS FOR WASHING SMALL PIECES OF TISSUE

So many different devices for washing tissues have been described that it seems almost superfluous to add another. Yet practically all those previously described involve the use of large quantities of running tap-water and mechanical means, such as netting, to prevent the escape, especially, of very small pieces of tissues. There are obvious disadvantages when tap-water is alkaline and the objects to be washed are small and require to be washed with other fluids than water or with solutions.

The apparatus shown in figure 6 is simple and easily set up in one way or another. Its value depends on the fact that large volumes of rapidly running water are not necessary, but that it is sufficient to have a slow change, provided the volume to be changed is not great. The last condition is obtained by putting the tissue in a small container which it nearly fills. Then all that is needed is to supply a small stream at the bottom of the container as near the tissue as possible. Such a slow stream washes as effectively as a rapid one and is easily obtained by means of a capillary siphon made entirely of glass or in part of fine capillary rubber tubing. With this method, distilled water, alcohol, or solutions of various kinds can be used.

For convenience the supply bottle can be set on a central support and made to accommodate several siphons. The

tissue to be washed can be placed in small glass cups made by cutting off the lower ends of vials of appropriate size, and the latter can be placed in the funnels of thistle tubes which receive the overflow. The position of the cups is adjusted by moving the thistle tube up or down through the stopper of the waste bottle. The rate of flow of the fluid is determined by the dimensions of the siphon as described in the preceding articles.

APPARATUS FOR INJECTING THE VASCULAR SYSTEM WITH FIXING FLUID

The method of fixing tissues by forcing fixing fluid into the vascular system of an animal has long been known and used. By it material can be obtained more perfectly preserved than in any other way, because the fixing fluid is brought instantly into contact with practically all of the cells at the same time—obviously, a great advantage in the case of fluids having poor penetrating qualities.

In employing this method various kinds of apparatus may be used. For occasional use a glass Luer syringe is excellent, but troublesome when much material is to be handled. A simple easily constructed apparatus has been described by Allen ('19). The writer has for several years used an apparatus substantially like the one here described which is simple and easily operated.

It consists of two receptacles of about 300 cc. capacity (fig. 7) attached to an upright support, furnished with outlets at the bottom, side necks near the top, and at the upper ends stoppered necks for filling. The bottom outlets are connected by means of rubber tubing with the paired tubes of a two-way stopcock, the single tube being in turn connected with the slender rubber tube attached to the cannula. One of the receptables is usually filled with physiological salt solution, the other with the fixing fluid.

The side necks of the receptables are connected with the source of compressed air. In the absence of better means of obtaining the latter, the writer uses a stout tank of about 5

gallons' capacity to which are attached a pressure gauge, a stopcock, and an automobile tire valve, to which may be attached a tire pump with which any necessary pressure can

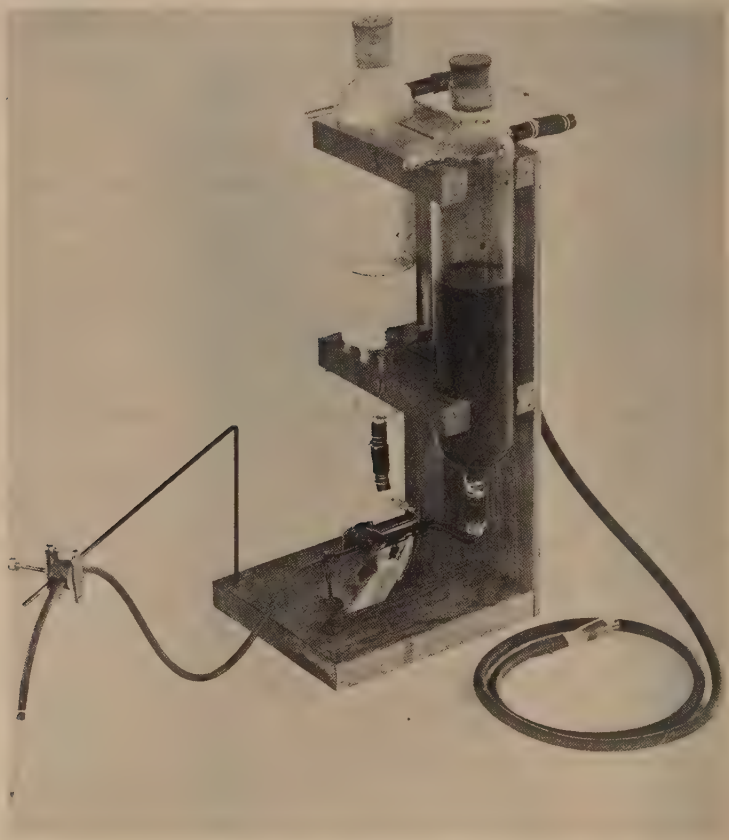


Figure 7

easily be obtained. Ordinarily about 15 pounds per square inch is sufficient.

For operating the apparatus the receptacles are filled and all tubing leading from them emptied of air by running first fixing fluid just beyond the stopcock and then salt solution

sufficient to wash all fixing fluid from the rubber tube to the tip of the cannula. It is a good plan to put a pinchcock on the tube near the cannula to prevent air or blood entering while the latter is being inserted in the blood vessel selected. After the desired amount of salt solution has been injected, it may be followed instantly with fixing fluid by turning the stopcock.

It has been the writer's experience in fixing rats that on the whole the best results are obtained by injecting through the abdominal aorta distal to the kidney either posteriorly (for female reproduction organs) or anteriorly (for the testis as well as anterior part of the body), for the reason that the breathing and anesthesia are not interfered with, thus making it possible to keep the animal alive up to the time of fixing. Equally good results follow injection through a carotid. This has the advantage that most of the blood may first be allowed to escape.

A DEVICE FOR FACILITATING THE INFILTRATING OF TISSUE WITH PARAFFIN

It is the practice in several laboratories to use ordinary electric lamps, with shades or reflectors, supported over watch-glasses or other vessels, to keep paraffin at the melting-point. As far as the writer knows, the idea was first suggested by McClendon ('08) (see also Guyer, Animal Micrology, p. 14). The method has been found to be more satisfactory than the use of water-baths of various designs. It is particularly appropriate for class work, enabling students to do their infiltrating and embedding on their own tables, thus avoiding congestion and confusion about a large common infiltrating apparatus.

The temperature of the paraffin can be kept at the melting-point by adjusting the height of the lamp above a ring of six to eight Syracuse watch-glasses so that at the outer sides of each dish the paraffin is not quite fluid. To avoid the ill effects of air currents, a piece of cardboard or metal may be bent and set so as to enclose the dishes and lamp shade.

Some individuals have used a small tin pail over which the lamp is placed, the watch-glasses being on the bottom. But as this arrangement, like that of having the watch-glasses on the table, is awkward, the following apparatus may be found more convenient.

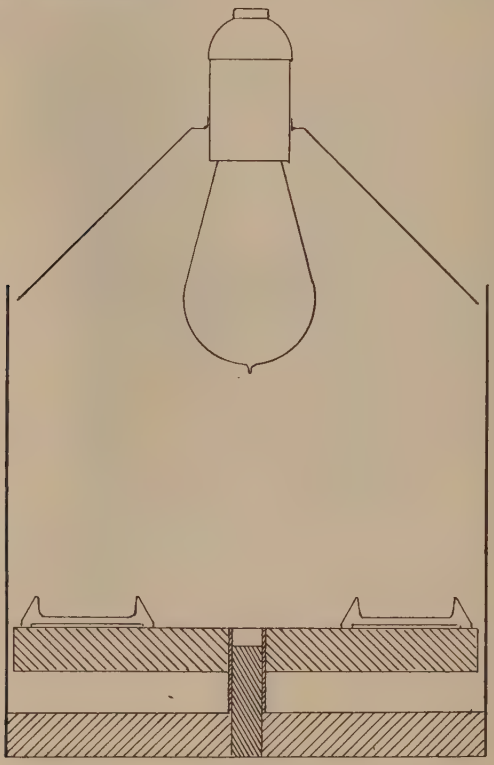


Figure 8

It consists of a circular wooden disc about 8 inches in diameter supported as shown in section in figure 8 so that it rotates freely in a horizontal plane about its center. The supporting post is fixed to the center of another disc about $\frac{1}{4}$ inch larger, which forms a base. To the periphery of the base is fastened a bent piece of stiff cardboard about 8 inches

high so as to form an enclosure about the rotating disc and lamp shade on all sides except for an opening about 4 or 5 inches wide. The opening is toward the worker and may be closed by a smaller piece of bent cardboard which may be slid to one side to give access to the interior. By means of the rotating disc on which the watch-glasses or other receptacles are placed, it is easy to bring any one of the watch-glasses to the front without disturbing the others or lowering the temperature greatly.

The conical shade, and ordinary cord socket, can be supported on an iron stand and brought over the watch-glasses. It should be of nearly the same diameter as the upper disc. The larger the lamp used, the higher it must be placed and the more uniform the distribution of its heat will be. Metal can be used in place of cardboard.

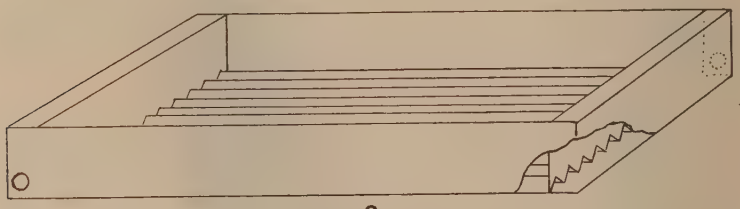
The source of heat is also a source of light—a consideration of importance in the orienting of objects in embedding.

AN IMPROVED PAN FOR THE POURING OF WAX PLATES

This is intended merely to accelerate the well-known process in which a measured quantity of melted wax is poured on the surface of a large pan of hot water and allowed to cool. It consists of a pan 3×4 feet having a double bottom, the inner one of which is made of corrugated galvanized iron (fig. 9) riveted to the outer bottom. The downward directed grooves open at both ends into chambers extending completely across the ends of the tray. One of these chambers is connected both with steam and cold-water systems so that either can be caused to flow between the bottoms. The other chamber has a movable connection to a drain pipe, so that by lifting or lowering the outlet pipe the level of water in the end chambers can be regulated.

In operation enough water is run into the pan to cover the highest ridges of the inner bottom to a depth of about $\frac{1}{8}$ inch; steam is then admitted between the bottoms until the water reaches a temperature considerably below boiling (about 75°C.) when the proper quantity of wax heated just to the

melting-point is distributed over the surface by means of the funnel shown in figure 10. The funnel is supported over the pan in such a way that it can be moved back and forth over this whole area with its outlet just at the surface of the water. Air bubbles can be removed from the layer of wax floating



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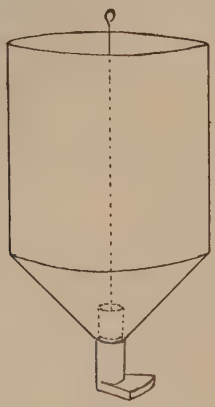

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Figures 9 and 10

on the water by playing a bunsen-burner flame on it, but if the temperature is not too high few bubbles will be formed. When the wax has come to equilibrium, the pan should then be covered to prevent air currents from disturbing the wax and cold water run through the double bottom slowly enough not to cause vibrations until the wax congeals.

AN ELECTRICALLY OPERATED MACHINE FOR CUTTING WAX PLATES FOR MODELS

The photograph of the machine shown in figure 11 gives the general appearance and arrangement of parts. The essentials consist of an electrically heated nichrome wire, given a vertically reciprocal motion, and a nozzle through which the wire passes, the nozzle serving to remove by suction the wax melted by the wire as the plate of wax is moved against the wire. The principle is the same as in the

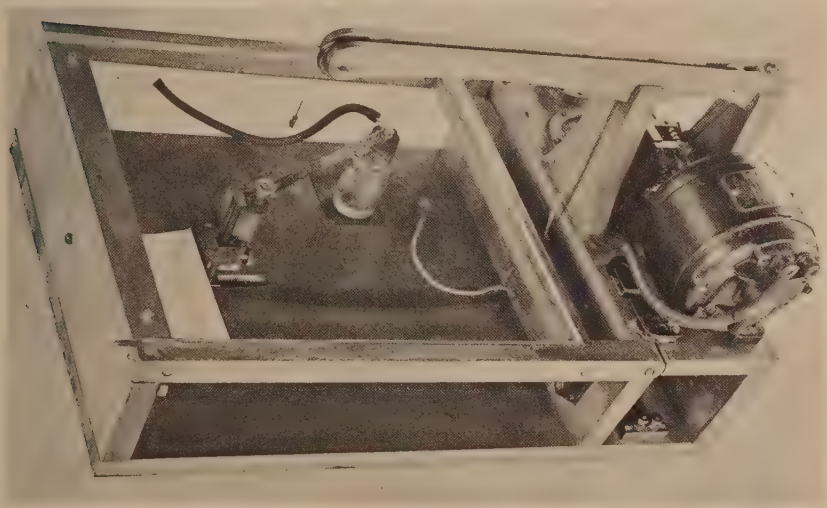


Figure 11

machine described by Mark ('07). The motion of the wire is imparted by a motor operating through a worm gear and crank from which a cord passes over two pulleys to a small clamp holding the upper end of the wire. The latter is kept taught by a weight, which slides up and down on a guide post, below the nozzle. The weight carries a convenient clamp for holding the wire.

The nozzle is shown in section in figure 12. It will be seen that the wire (w) (of 29 standard gauge) is held at the

center of the opening of the nozzle by a steel guide (*sg*), so that the wax plate can be moved against the wire in any direction. By means of the adjusting nuts, *n*, *n*, the nozzle can be raised or lowered through the opening in the glass plate (*g*) so that it barely touches the under side of the wax plate, causing all the air entering it to pass through the slot cut by the hot wire. The melted wax is carried off through a short piece of copper tubing covered with a thin layer of

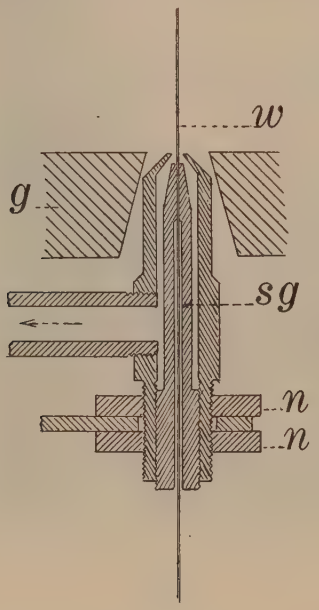


Figure 12

asbestos and electrically heated by a single turn of wire about it, and caught in the trap (fig. 11) which consists of a bottle. Suction can be obtained in any convenient way.

The wire must be perfectly straight—a condition easily obtained by gently stretching it with pincers before putting it in the machine.

The heating current is led in series through the wire heating the copper tube attached to the nozzle, the cutting wire,

and a small resistance. The nozzle is kept sufficiently warm by both the cutting wire and the copper tube.

It will be noted that the wax plate to be cut lies on a piece of plate glass pierced by a hole through which the nozzle and cutting wire pass. The glass lies in a wooden frame hinged at the side next to the motor so that the side near the operator may be raised a short distance by means of a simple lever. Raising the glass has the effect of lowering the nozzle, so that melted wax is not drawn out of the wire cut, but remains and congeals, the operator thus being enabled to begin actual cutting at any part of the wax plate.

Some of the advantages of this method over the ordinary one of cutting with a knife lie in the rapidity with which the operation may be carried on, in the fact that the edges on both sides of the cut are clean, and in the absence of injury to small pieces of wax.

Ordinary carbon-paper will leave no tracing on wax. It can, however, be made to leave a heavy black line if it is first immersed in xylol and then dried.

A BATH FOR SPREADING SECTIONS

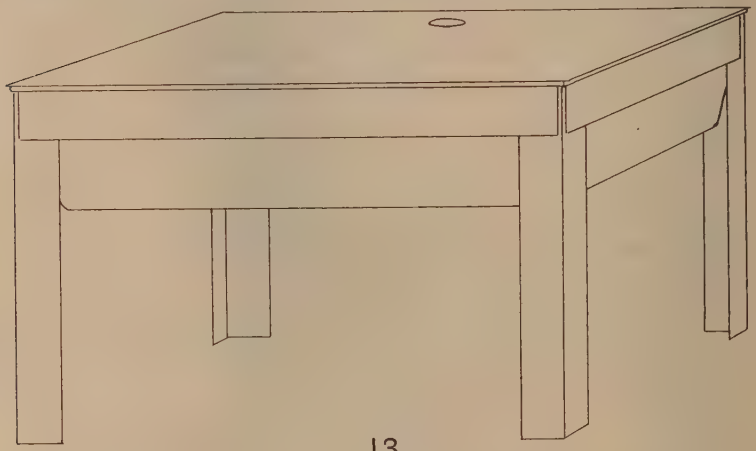
A very satisfactory, inexpensive, and serviceable water-bath can be made by mounting an ordinary granite pan $10 \times 6 \times 2$ inches on a frame about 6 inches high, made of galvanized iron, as shown in figure 13. The pan is covered with a perfectly flat piece of galvanized iron pierced near one edge by a hole large enough for a cork stopper through which is thrust a thermometer. The temperature is easily controlled and maintained with sufficient constancy by an alcohol lamp which, besides having the size of its flame adjusted, may be pushed under the pan and removed as required. By this means slides are not only kept uniformly warm while the sections are expanding and being pushed into position, but may also be dried.

To facilitate seeing unstained sections, the whole or part of the upper surface of the cover may be blackened.

In case the top tends to become uneven by warping, angles of sheet-iron can be soldered to its under surface.

INSTRUMENTS FOR DISSECTING MINUTE OBJECTS

In view of the great difficulty, if not impossibility, of putting sharp edges on slender, needle-like instruments, keen enough to cut delicate objects neatly, the writer hit upon the scheme of making such instruments from sharp safety-razor blades. Various forms such as herewith illustrated (fig. 14)



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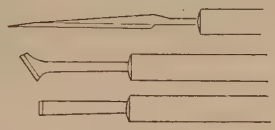

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Figures 13 and 14

and other desired forms can easily be fashioned. The blades are so hard that they may be broken, and the line of breaking controlled by clamping them in a vise so that the line along which they are to break coincides with the edges of the jaws of the vise. The crack will take place along the whole length of the line if a block of wood is forced evenly against one side of the protruding blade as though it were hinged at the line in question.

In this way the instrument can be roughly blocked out. Final trimming is done by holding again in a vise and grinding on an emery wheel down to the jaws of the vise. This makes it possible to grind slender pieces without either breaking them or drawing the temper. The shank may be made

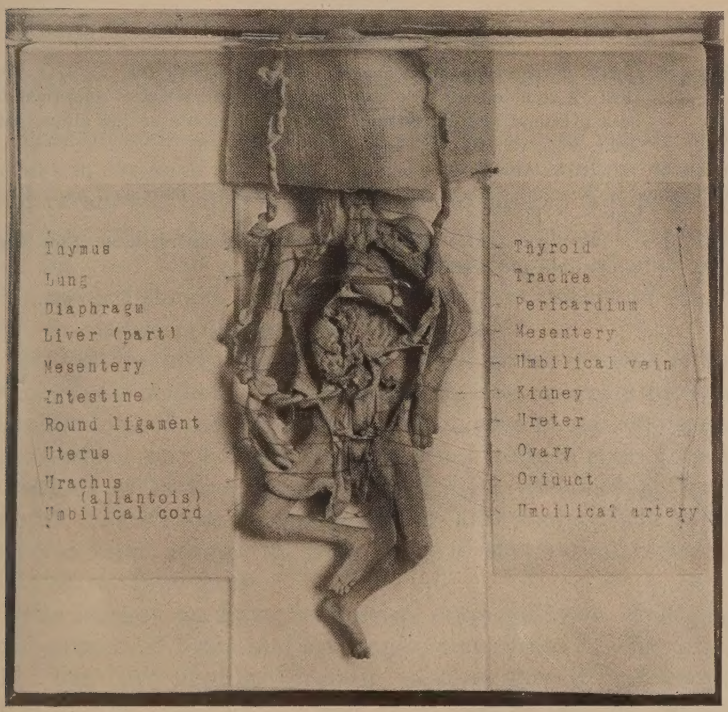

Figure 15

slender enough to force into an ordinary wooden needle holder. The blade may be so inserted by holding it in a vise with only a small portion protruding at a time and driving the handle or holder onto it with light taps.

With care the edge can be kept perfect during the processes just described. Instead of resharpening, dull instruments are more easily replaced by new.

ON LABELING DEMONSTRATION DISSECTIONS

The accompanying photograph (fig. 15) sufficiently illustrates the use of fine threads attached to the parts to be named on the one hand, and on the other to the labels or lists of structures.

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A METHOD FOR SECTIONING THE WHOLE HUMAN BRAIN

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The methods of sectioning the whole human brain heretofore in use have required a great length of time for celloidin infiltration and more difficulty in sectioning and tracing nerve pathways than the parowax method which we have been using for the past four years. It therefore seems desirable that this method should be recorded for the convenience of others who are teaching the anatomy of the brain.

By this method it is very easy to get serial sections of the whole brain without discouraging difficulty or waste of time. The sections of the whole human brain give about 2000 specimens which may be used for individual work in class demonstrations or in tracing nerve pathways. Often every tenth section is sufficient for preservation and study. The whole process from autopsy to completion of the sections requires only thirty-four days.

The method we have been using is as follows: Take any well-preserved brain and thoroughly dehydrate it. The brain that has been kept in formalin solution should first be put in 60 per cent alcohol for four days, then 80 per cent alcohol for four days, 95 per cent alcohol for four days, absolute alcohol for seven days.

The brain is allowed to drain for a short period, approximately two hours, allowing the alcohol to drain out of the ventricles. It is then immersed in parowax and allowed to remain for six days. It will take about eight pounds of parowax, which should be kept at a temperature not under 50° nor over 60°C. The whole mass is then allowed to cool for twenty-four hours. With a bunsen burner warm the

outer walls of the container and allow the whole mass to come out. It is then shaped into a block about $6 \times 8 \times 8$ inches. This block of parowax containing the brain is then attached to a block of wood $2 \times 8 \times 8$ inches.

The equipment necessary for the work is as follows: The rotary type of slicing machine, manufactured by the United States Slicing Machine Company. It is necessary to make some minor changes in it. First have a good mechanic make a much smaller feed screw, that is, with closer threads. Attach a small razor blade on each side of the rotary blade to keep the parowax from adhering to it.

The block is put in the machine and clamped with the holder. Warm the block of parowax with the bunsen burner, then start the machine and cut until the sections become brittle. The whole mass must be warmed to get the best results, especially in winter.

As sections are cut they should be mounted on slides 5×7 inches. This is best done by having a container about 24×30 inches, 2 inches deep, containing water which should be warm. Place the section in the water and gently push the slide under it with one side tilted up, pressing the section down at one corner; then lift it out of the water. The excess parowax is gently pulled away from the outer edge of the brain, allowing only that part of parowax to remain that has infiltrated the brain.

The slide is then put on a table and a soft cotton cloth spread over it and gently pressed until the excess water is absorbed. Remove cloth, put slide away, and allow to dry. The parowax which has infiltrated the brain will preserve it without any cover for years.

With this method it is possible to section the whole brain in sections less than 50 μ.

Preparations of brain prepared as above described show very well, without staining, the anatomic relations of the chief brain divisions, the basal ganglia, internal capsule, the ventricles, choroid plexus, and many of the fiber tracts, such as the olfactory, optic, etc. The details of the medulla stand out fairly clearly, so that the relations, size, etc., may be readily studied by the student.